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Zjištění závislosti mezi srdeční variabilitou a změnou průměru zornice

Quantification of the Relation between Pupillary Diameter and Cardiac Variability

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1. Research on the methods of the measurement of pupillary diameter oscillations and heart rate measurements.
2. Realization of a biofeedback experiment to enslave the brightness of a light signal to the oscillations of the pupil. Together with the eye signal will be recorded the electrocardiogram.
3. Development and realization of cardiac variability over short time periods. Analysis over measured values to find relation between them.
4. Evaluation of analysis results and comparison to hypothesis, there is expected strong relation between these two types of signals as both are under the influence of the sympathetic nervous system.
5. Evaluation of thesis results.

References:

[1] LAMIREL, Cedric, Suzon AJASSE, Antoine MOULIGNIER et al. A novel method of inducing endogenous pupil oscillations to detect patients with unilateral optic neuritis. *PLoS ONE.* 2018, 13(8): e0201730. ISSN 1932-6203. DOI: 10.1371/journal.pone.0201730.
[2] CLARKE, Robert J., Hongyu ZHANG a Paul D. R. GAMLIN. Primate Pupillary Light Reflex: Receptive Field Characteristics of Pretectal Luminance Neurons. *Journal of Neurophysiology.* 2003, 89(6), 3168-3178. ISSN 0022-3077. DOI: 10.1152/jn.01130.2002.
[3] PARNANDI, Avinash a Ricardo GUTIERREZ-OSUNA. Contactless Measurement of Heart Rate Variability from Pupillary Fluctuations. In: *2013 Humaine Association Conference on Affective Computing and Intelligent Interaction,* Geneva, Switzerland. IEEE, 2013, s. 191-196. EISSN: 2156-8111. DOI: 10.1109/ACII.2013.38

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Prohlášení studenta

„Prohlašuji, že jsem tuto diplomovou práci vypracovala samostatně. Uvedla jsem všechny literární prameny a publikace, ze kterých jsem čerpala.“

V Ostravě dne 15. 5. 2020

Abstrakt

Diplomová práce se zaměřuje na měření velikosti zornic společně s měřením srdeční aktivity a na hledání vzájemného vztahu mezi těmito dvěma signály. Existuje hypotéza, která předpokládá silný vztah mezi těmito signály, jelikož jsou oba inervovány sympatickým a parasympatickým nervovým systémem. Během zpětnovazebního experimentu, založeného na eye-trackingu, se jas promítaného stimulu mění v závislosti na aktuální velikosti zornice a vznikají oscilace zornic. EKG signál je snímán po celou dobu tohoto experimentu.

Pro analýzu dat je navržena aplikace v programu Matlab. Oscilace zornic a EKG signál jsou vyobrazeny v časové i frekvenční doméně. Zobrazení v časově-frekvenční doméně je zprostředkováno vykreslením srdeční variability a variability velikosti zornic. Analýza naměřených oscilací poskytla hodnoty pro porovnání s teoretickými znalostmi o srdeční variabilitě.

Klíčová slova

Oscilace zornic, variabilita oscilací zornic, srdeční variabilita, eye-tracking

Abstract

The diploma thesis is dedicated to measurement of the pupil size together with cardiac activity and to finding of the relationship between these two signals. There is hypothesis which expects strong relation between these signals as both are innervated by sympathetic and parasympathetic nervous system. In the biofeedback experiment based on eye-tracking is the luminance value of stimulus directed by real time pupil size and the pupil oscillations are generated. The ECG signal is monitored during the experiment.

For data analysis was designed an application in Matlab. The application presents pupil and ECG data in time and frequency domain. The pupil oscillation variability and heart rate variability are computed for processing in time-frequency domain. The analysis is mainly in frequency domain and using statistical analyses are obtained values to compare with the theoretical heart rate values.

Key word

Pupil oscillation, pupil oscillation variability, heart rate variability, eye-tracking

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List of used symbols and abbreviations

ANS	Autonomous nervous system
BPV	Blood Pressure variability
ECG	Electrocardiogram
FFT	Fast Fourier Transformation
fps	Frames per second
H_0	Null hypothesis
H_A	Alternative hypothesis
HF	High frequency
HRV	Heart rate variability
L	Luminance in digital value
LF	Low frequency
L_{max}	Minimum level of luminance
L_{min}	Maximum level of luminance
ON	Optic neuritis
P_a	Real time pupil size
P_{amax}	Pupil size corresponding to maximum level of luminance
P_{amin}	Pupil size corresponding to minimum level of luminance
PCT	Pupil cycle time
PLR	Pupillary light reflex
PO	Pupil oscillation
PSV	Pupil size variability

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1 Introduction

The aim of the thesis is to find a relationship between pupil size and cardiac activity to confirm the hypothesis which expects strong relation between these two signals as both are innervated by sympathetic and parasympathetic nervous systems. The muscle responsible of increasing of the pupil is mostly innervated by the parasympathetic nervous system and the dilatation is done by the sympathetic. Similarly, stimulation of the parasympathetic nerves reduces the heart rate and the sympathetic stimulation increases it. The pupil size and heart rate are determined by the balance between these two systems.

The first part of the thesis is dedicated to the research of previous studies on the topic of measuring pupil size and pupil oscillations and then of measuring this signal together with cardiac activity. Frequency of pupil oscillation was chosen as a good indicator of the condition of nerve fibres. The previous studies [6, 9] prove that the frequency is significantly lower for example in patients with optic neuritis.

In the practical part it was designed an experimental protocol for measuring the pupil oscillation and cardiac activity together. The pupil oscillation is obtained by a biofeedback experiment where the luminance of the stimulus displayed to the tested subject depends on the actual pupil size at every moment. The cardiac activity is monitored by ECG synchronized with the eye-tracker. To allow the comparison of these two signals it is computed pupil oscillation variability and heart rate variability.

The measurement is dedicated specially to the pupil oscillation to provide more information about the signal. It is analyzed mainly in frequency domain and using statistical analyses are obtained values to compare with the theoretical heart rate variability values.

2 Anatomy of the human eye and the pupil

The human eye is a specialized sense organ which is receiving visual images. The anatomy structure of the human eye is described in the Figure 1. The pupil is a circular hole in the iris. The iris, ciliate body and choroid forms middle layer of the eye called uvea. Its function is to nourish the eyeball. The iris also forms a kind of partition between anterior and posterior eye chamber. The pupil connects these two chambers. The size of the pupil is variable, between 2 and 8 mm. When the pupil is narrowed it is called miosis, when is dilated it is called mydriasis [3].

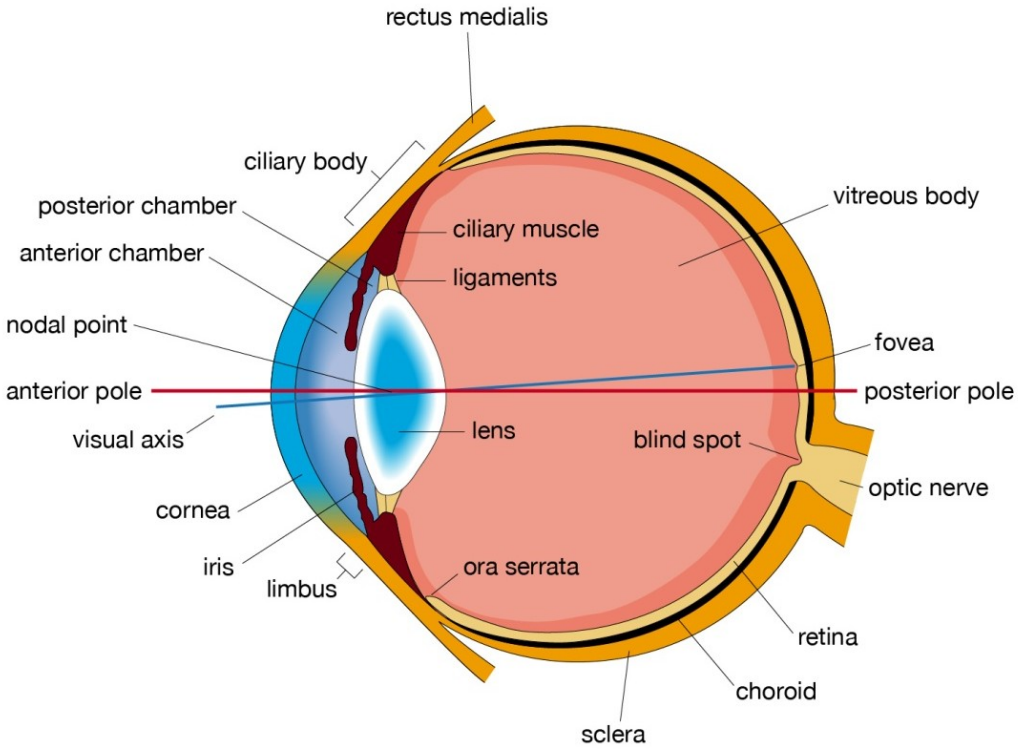

Figure 1: Anatomy of the human eye [1]

The main function of the pupil is to regulate the amount of light incident on the retina. After passing a beam of light through the optical environments of the cornea and aqueous humor, the pupil transmits a quantum of light for optimal imaging. If the intensity of light is too large, pupil miosis prevents from glare and to the other side, if the amount of light falling to the eye is small, the pupil increases its diameter [1].

The pupil size affects depth of field too. Narrowing the pupil increases it because it eliminates rays incident at a large angle. The elimination of peripheral rays as well removes a spherical and a chromatic defect of the optical system of the eye. The diameter of the pupil can be affected not only by light, but also by accommodation at close range, a painful stimulus, emotions, or chemically by some drugs [1].

2.1 Iris muscles affecting pupil size

The changing of the pupil diameter is ensured by two smooth muscles of the iris: musculus sphincter pupillae and musculus dilator pupillae (Figure 2). The pupil sphincter is made of circulary arranged muscle fibres with a width of 1 mm and it is located closer to the pupil edge. Vessels and binder run through it. In light colored iris the pupil sphincter is visible as thin brown hem. The pupil dilator extends at the root of the iris, it ends approximately 2 mm from the pupil margin. The pupil dilator lies deeper in the stroma than the sphincter and partially interferes into the ciliary body [1].

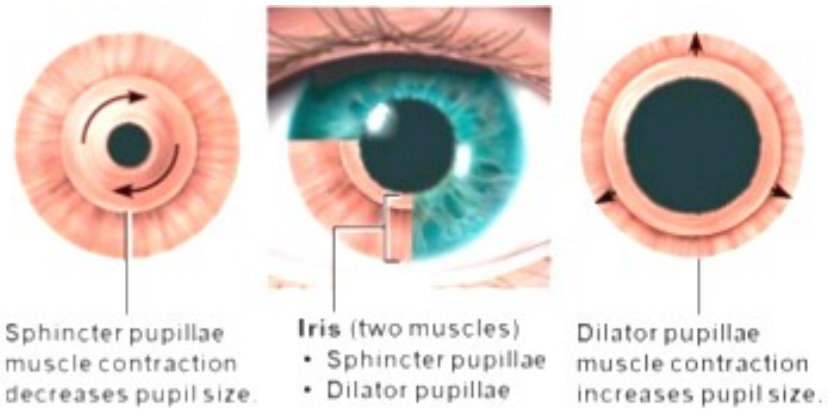

Figure 2: Iris muscles controlling the pupil diameter [2]

2.2 Innervation of the iris muscles

The muscles affecting the contraction and dilatation of the pupil are innervated by the autonomic (vegetative) nervous system. This system transfers excitement between the central nervous system and smooth muscle tissue and its purpose is to ensure the surviving of the body. In general, sympathetic innervation prevails during stress reactions of the organism, when the release of energy and rapid response is needed, while parasympathetic innervation predominates when the organism is at rest. The general manifestations of the parasympathetic activity on the organism are slowing of the heart activity, narrowing of the bronchi or increasing peristalsis of the digestive tract. The sympathetic system manifests itself in the organism with an increased heart rate, increased blood pressure, bronchoconstriction or increased hepatic metabolism [3].

The contraction of the pupil sphincter is enabled by the parasympathetic system, the pupil dilator is activated by the sympathetic system. Both muscles are antagonistic, so they have antagonistic innervation. During parasympathetic activity the eyes are accommodated to close, while during sympathetic activity the eyes are accommodated into the distance. This is due to the interconnection of the nerve pathways of the iris muscles that affect contraction of the pupil and muscle of the ciliary body affecting the contraction of the suspension apparatus of lenses [2, 3].

2.2.1 Parasympathetic system

Acetylcholine acts as a mediator for parasympathetic nerve fibres. Once acetylcholine is released, action potentials arise. The action of acetylcholine is deleted by hydrolysis because of the action of the cholinesterase in the tissue. Acetylcholinesterase is contained not only in the pupil sphincter, but also in the ciliary muscle, corneal epithelium, retina and choroid.

The innervation of the pupil sphincter is made by 3rd pair of cranial nerve, n. oculomotor. The parasympathetic fibres that bring nerve impulses to the sphincter are centered in the Edinger-Westphal nucleus, which is located in the mesencephalon anteriorly and upward from the main nucleus of n. oculomotii. The front part of the core is the center of photoreaction, the back part controls the accommodation [2, 3].

2.2.2 Sympathetic system

Noradrenaline acts as a mediator for sympathetic nerve fibres. In the pupil dilator we can find α-1 and α-2 receptors. Activation of α-1 receptor causes active contraction. The irritation of α-2 receptors inhibits presynaptic terminals and prevents absorption of noradrenaline. The result is an indirect activation of the dilator. The rest of noradrenaline is deleted in the tissue by the enzyme catechol-o-methyltransferase.

The innervation of the pupil dilator is made through the cervical sympathetic system. The dilation center accepts signals from the hypothalamus and lies in the Budge center at the interface of the cervical and thoracic spinal cord. In addition the sympathetic fibres innervate also blood vessels [3].

2.3 Nerve pathway of pupillary light reflex

When light falls on the retinal photoreceptors, the parasympathetic nerve is activated, its fibres conduct an impulse from there to the brain (afferent pathway) and after evaluating, the signal descend into the orbit to activate the pupil sphincter (efferent pathway). On the contrary, if small amount of light falls on the rods and cones, the sympathetic system is activated and the pupil dilator dilates the pupil [3].

2.3.1 Parasympathetic afferent pathway

The reaction to the light begins with an analysis of the amount of light by the rods and cones in the retina. Here the yellow spot show the greatest activity. The first section of the path goes together with the optic pathway (2nd optic nerve). 20% of the visual nerve fibres are the fibres of the afferent pathway, specifically small cell axons called W-cell. Fibres from the same half of the retina of the both eyes always go through the optical tract. The right tract fibers from the temporal part of the retina of the right eye and the nasal part of the retina of the left eye, it means fibers from the right halves of the retina.

The nerve fibres leave the visual pathway before the corpus geniculatum laterale and avoid synapses. Then the fibres goes medially through the brachium colliuli superioris to the pretectalis area next to mesencephalon. Pretectal nuclei is connected by neurons to each other and also to the equilateral and bilateral Edinger-Westphall core. Then the pathway continues in two branches to E.W. core: one branch goes directly to the unilateral core, the second goes way of the rear commissure to the contralateral nucleus. The fact that the afferent parasympathetic pathway crosses twice causes narrowing pupils of the both eyes even if only one eye is exposed. This is called consensual reflection of the other eye [3].

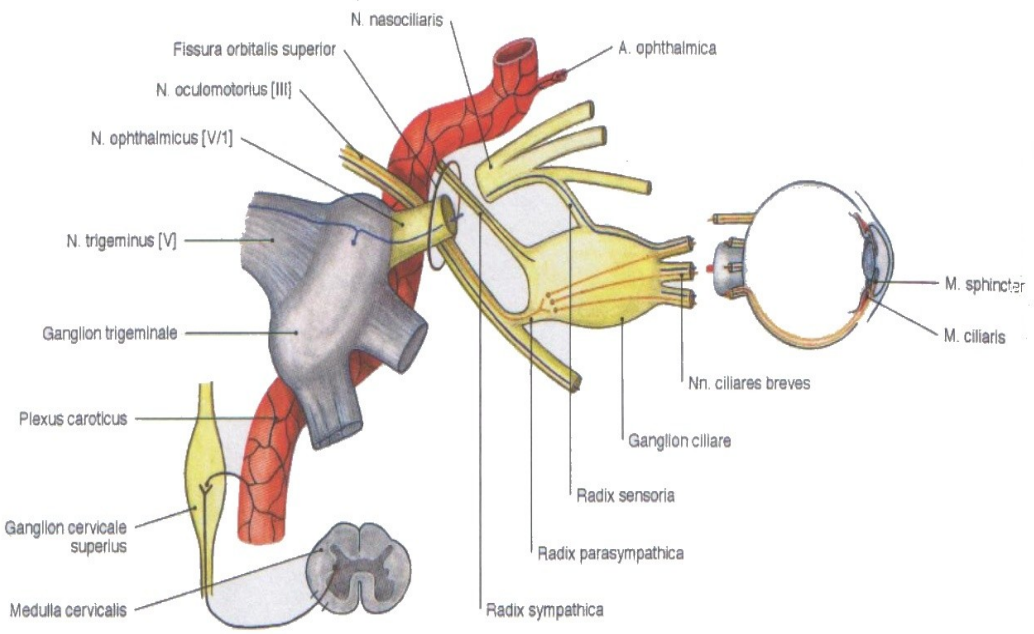

Figure 3: Parasympathetic afferent pathway [3]

2.3.2 Parasympathetic efferent pathway

The descending part of the reaction to light is two-neuron and partly follows the path of n. oculomotorius (3rd cranial nerve). The path is common to the pupil sphincter and ciliary muscle. The first neuron connects E.W. nucleus with ciliary ganglion (this represent connection between the mesencephalon and the orbit). The second neuron connects the orbit and pupil sphincter. Pupilomotor fibres are located in the dorsomedial part of n. oculomotii [3].

Reflex nerve fibres for photoreaction after leaving E.W. nuclei meet motor fibres passing through the red nuclei. They leave the brainstem as a stain of oculomotorii in the interpedunular fossa and then stand out through hard diapers nearby posterior processus clinoideus. They continue along the cranial base to the sinus cavernous and they are going through its outer layer into the orbit through the fissure orbitalis superior. The fibres reach the ciliary ganglion at the tip of the orbit where they

interpolate. From there nn. ciliares breves continue through sclera and choroidal space to the pupil sphincter. Only 3% of the nerve fibers reach the pupil sphincter, the rest of fibers continue to the ciliary muscle [2, 3].

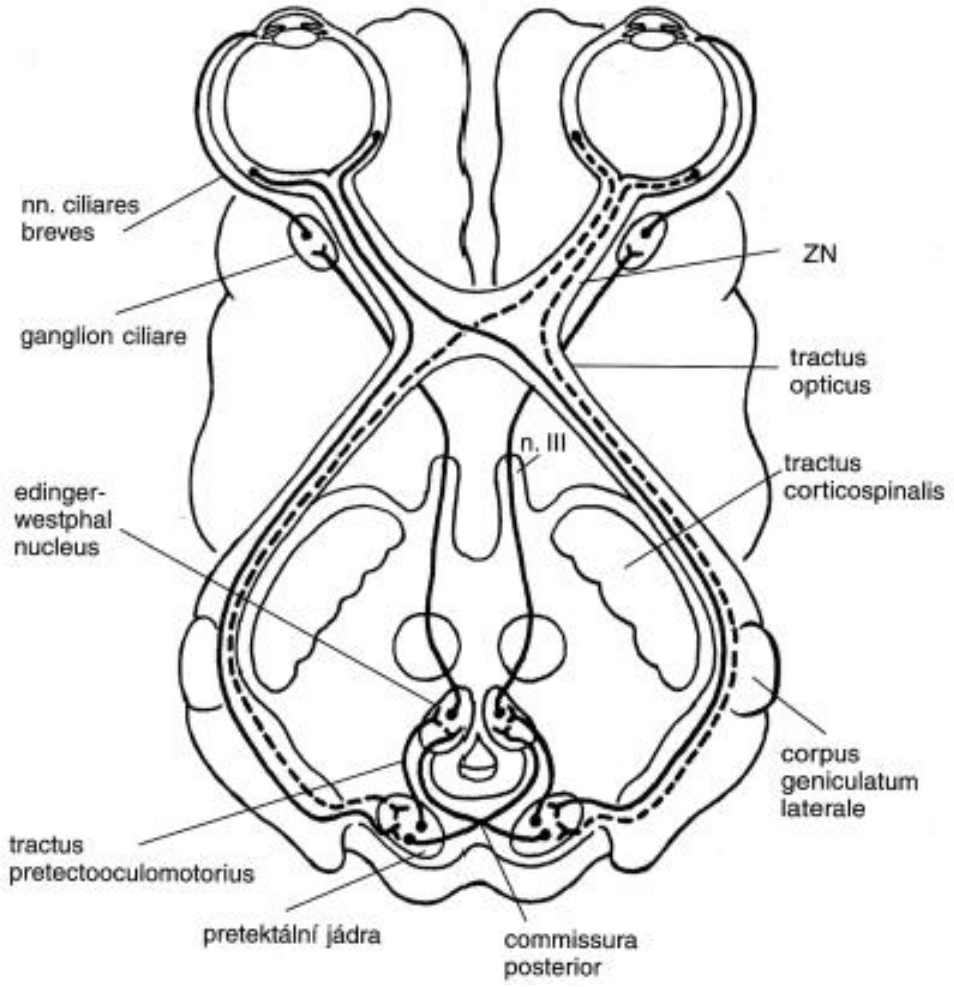

Figure 4: Pathway of pupillary light reflex [3]

2.3.3 Sympathetic efferent pathway

The efferent nerve pathway responding to the loss of light in the retina is made by three neurons. The first neuron connects the hypothalamus and the ciliospinal Budge center. The second neuron connects this center with ganglion cervical superius in the cervical sympathetic nerve. The sympathetic fibers enter to the orbit through two branches. The first goes along a. carotis interna to the Gasse semilunar ganglia and then the dilator. The second goes through plexus caroticus cvernosus to the fissure orbitalis superior and then goes the way of nn. ciliares breves to the pupil dilator. The sympathetic reflex path does not cross anywhere, so if it is damaged, it will take effect only on the damaged eye, not on both sides [1, 3].

3 Anatomy of the human heart

The heart is the main organ of the vascular system and its weight is up to 390 g. Its location extends into both parts of the thorax, which has a protective function. The heart is made by three layers: epicardium, myocardium (cardiac muscle) and endocardium. These layers have rich vascular supply. The epicardium is the inner leaf of the serous pericardium. The myocardium forms the main volume of the heart. Muscle bundles are long, circularly and spirally oriented muscle fibres. The endocardium is formed by an endothelial lining extended by a thin layer of connective tissue [11].

The heart is divided in two atria (atrium dextrum and atrium sinistrum) and two chambers (vetriculus dexter and vetriculus sinister). These cavities perform the function of the human pump. The cavities are separated apart by an interventricular septum. The atrium dextrum receives returning deoxygenated blood to the heart from the circulation. Blood is supplied through three veins: upper and lower vena cava and conoronary sinus. Blood flows from the atrium dextrum to the ventriculus dexter and from there the deoxygenated blood is inputted to the truncus pulmonaris, a large artery that carries blood to the lungs. Between the ventriculus dexter and pulmonary artery is the pulmonary valve. Oxygenated blood is supplied by right and left pulmonary veins to the left atrium. The mitral valve is located between left atrium and left ventricle. The left ventricle (vetriculus sinister) input blood into the body's circulation by aorta. Aorta valve is located between left ventricle and aorta [10, 11].

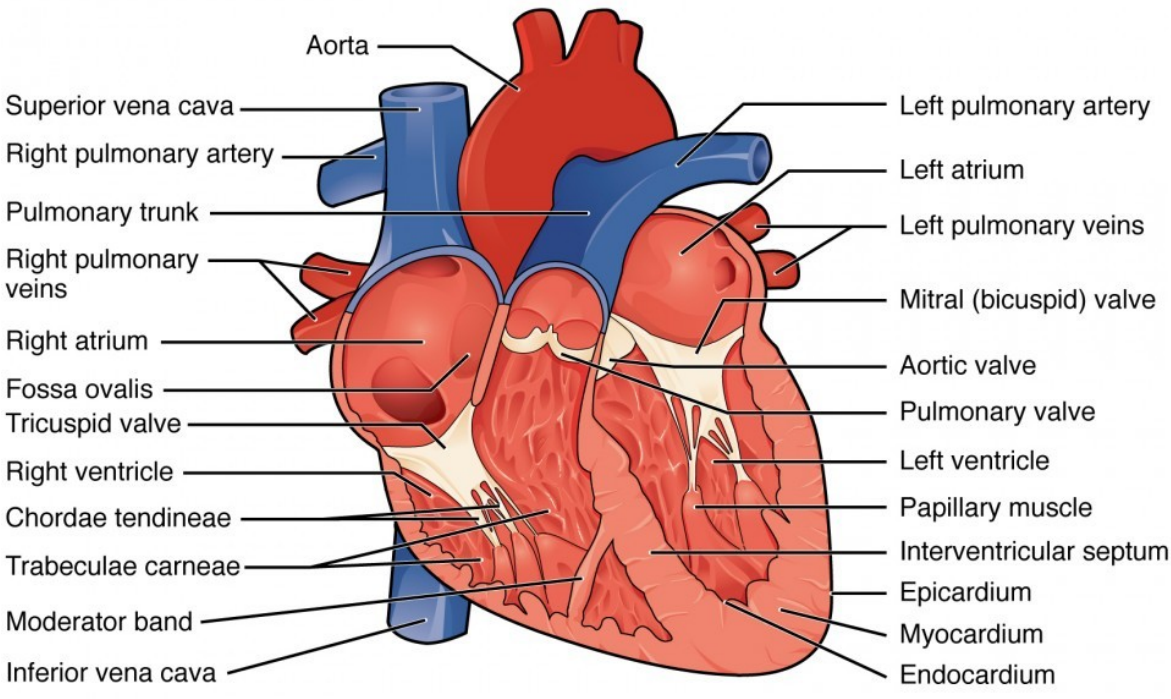

Figure 5: Anatomy of the human heart [10]

3.1 Innervation of the heart

The heat rhythm is determined by the activity of the sinoatrial node, but it can be altered by control of the autonomous nervous system. The nerves of the heart contain organ sensory fibres. Parasympathetic fibres slow down the heart rate and sympathetic fibres speed up the heart rate and also increase the strength of heart contractions. The autonomic nervous system is controlled by the cardiac centers in the spinal cord reticular formation. In the spinal cord it is situated the centre responsible of slowing down cardiac activity, a cardioinhibition center and a center responsible of accelerating cardiac activity. These centers are controlled by higher brain centers stored in the hypothalamus, the grey matter around the aqueduct and in the amygdale [11].

4 Eye-tracking and pupilometry

Eye-tracking is the method of measuring eye positions and eye movements, checking the point of gaze and also for measuring pupil size, so it can be the method of pupilometry. It can be used in different disciplines, from cognitive science, psychology and neurology, up to marketing research. Many methods of eye-tracking have been used throughout the history. Some of the methods of measuring eye movements are electrooculoprahy, magnetooculography or infrared oculography. This thesis was focused on videooculography, which consist in measuring the position of the distinguishable parts of the eye. It is mainly used detection of pupil shape, limbus position (border between cornea and sclera) and corneal reflection (reflection of light source from the cornea) [4].

Interpretation of eye movements may or may not be performed automatically and it can involve simple visual inspection of eye movements from video. However, manual video browsing is extremely time-consuming, error-prone and limited by the sampling frequency of the video device. Automatic limb tracking often involves the use of infrared photodiodes mounted on a frame around the eye. The advantage of monitoring the limbus is that the size of the limbus is not affected by different lighting conditions and it is stable. On the contrary, the disadvantage is the frequent covering of the limbus with eyelids.

The eye-tracker is used to be located under the monitor, on which the stimulus is displayed. Part of the device is one or more infrared lights that shine toward the subject. The device includes a camera that captures the subject's eyes. Based on image recognition is then found the center of the pupil and the corneal reflection of the infrared lights [4].

4.1 The EyeLink 1000 Plus Eye Tracker

To measure the pupil size in this thesis was used eye-tracker EyeLink 1000 Plus. It is a flexible video-based eye tracking system. The eye-tracker is capable of detecting changes in pupil size of 0.1% of the pupil diameter and with sampling frequency of 1000 Hz the system allows pupillary responses to be measured with very high details.

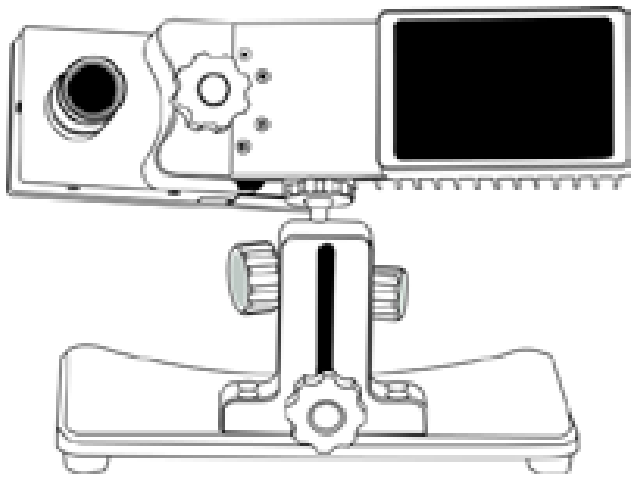

Figure 6: Desktop Mount EyeLink 1000 Plus [5]

For eye-tracking it was used the most popular mounting option, the Desktop Mount. It is situated just below the tracked stimulus, 40-70 cm from the subject to allow measuring without any electronics near to the subject's head. The system allows access eye data with a 2.2 ms delay, which is precise enough to use it for biofeedback experiment [5].

5 Theory of measuring cardiac activity

5.1 Electrocardiography

Electrocardiography (ECG) is a diagnostic method used for monitoring of electrical activity of heart. It is one of the most popular diagnostic methods. The 80% of human body is water, which has the ability to conduct electrical impulse and because of that we have the possibility to measure electrical potentials from human body surface. The ECG is usually monitored using noninvasive methods from the chest or from the limbs of the person [12].

5.1.1 Einthoven's limb leads (I, II, III)

Einthoven triangle is a connection using three electrodes located on the arms and left leg. The electrode on the right leg is not used for measurement, but as a neutral electrode and represents active ground that is connected to reduce positive deflection. The model of the triangle was invented by Willem Einthoven. In the past the electrodes were installed at the end of the limbs, but nowadays it is known that it can be located under clavicle or on shoulders. Precise location of the electrodes is not important as in precordial leads. There are three Einthoven's leads. Lead I connects LA an RA electrode, lead II connects LL and RA electrodes and lead III connects LL and LA electrodes [12].

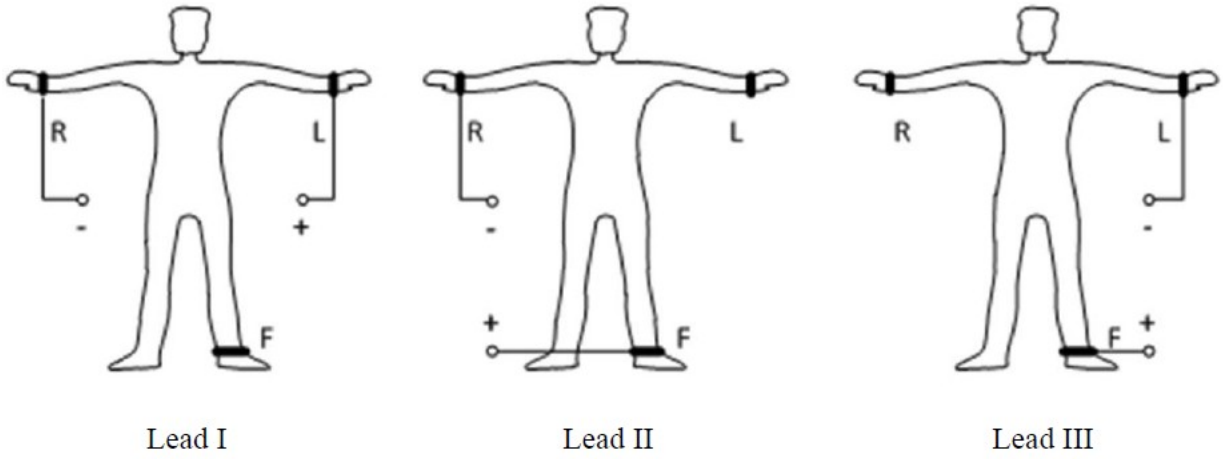

Figure 7: Einthoven's limb leads [12]

5.1.2 Goldberg's augmented limb leads (aVR, aVL, aVF)

Augmented limb leads use the same limb electrodes as in Einthoven's triangle, but the leads are different. One of the electrodes is used as positive terminal and the other two electrodes are connected trough two equal resistors to the reference terminal. In lead aVR the positive terminal is on the right hand, in lead aVL on the left hand and lead aVF on the left leg [12].

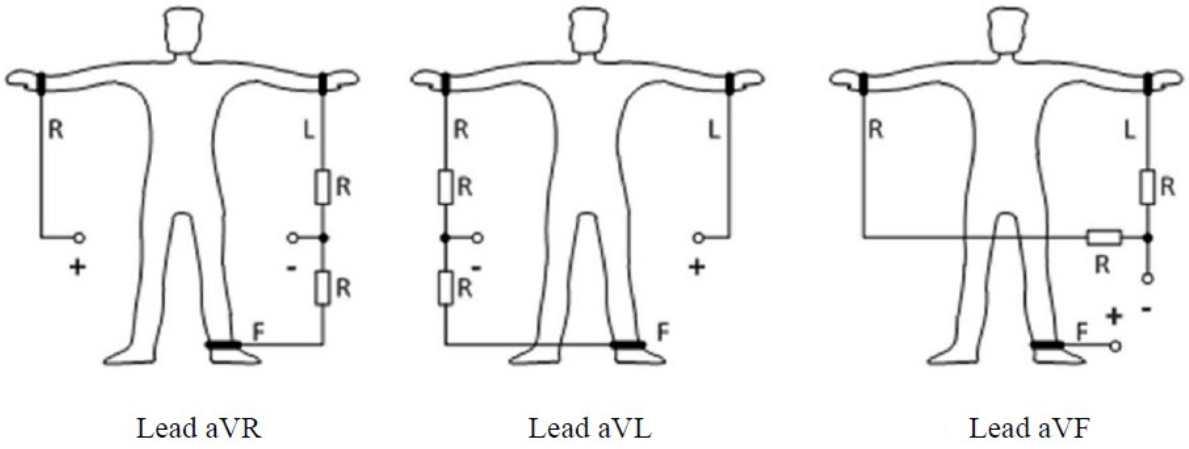

Figure 8: Augmented limb leads [12]

5.1.3 Precordial leads (V1, V2, V3, V4, V5, V6)

Precordial (chest) leads consist of a positive electrode placed on the chest. The position of the positive electrodes (V1, V2, V3, V4, V5, V6) is very important for a valid monitoring. Negative terminal is on the reference terminal called Wilsons's central terminal (WCT). WCT is formed by connecting limb electrode trough 3 resistors to the terminal [12].

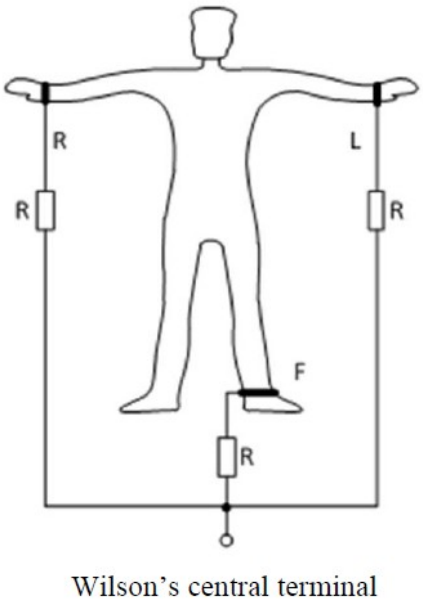

Figure 9: WCT used in precordial leads [12]

5.2 Methods for heart rate variability analysis

The heart rate variability can be analyzed by various mathematical methods of time series of R-R intervals. It is possible to analyze short-term records (short term variability, STV) or 24-hours ECG records (long term variability, LTV). Using of STV records is simpler and more comfortable for the tested subject. The disadvantage of STV is that captures only frequency components with period less than 1 minute. The time series is possible to process by analysis in time domain, using frequency (spectral) analysis or using other graphical methods. Time analysis is suitable for long records, its parameters inform about the size of HRV fluctuations. For analysis of short term records is mostly used frequency analysis [13].

5.2.1 Spectral analysis of HRV

In heart rate spectral analysis it is monitored heart rate in four frequency bands [13]:

- **High frequency band** (HF) with a frequency range of 0.15-0.4 Hz and 9-24 cycles per minute. Activity in this band reflects the effect of the respiration and is considered a major indicator of parasympathetic influence to cardiac activity.
- **Low frequency band** (LF) with a frequency range of 0.04-0.15 Hz and 2-9 cycles per minute. This band is affected by both components of autonomic nervous system. The LF component is increased by stimulation of sympathetic nerve fibres.
- **Very low frequency band** (VLF) with frequency range of 0.0033-0.04 Hz and 0.2-2 cycles per minute is under influence of vasomotor tone, which is associated with thermoregulation. The main activity is from sympathetic system.
- **Ultra low frequency band** (ULF) is apparently affected by physical activity.

6 Research of previous studies on pupilometry and measurements together with cardiac activity

Before designing my own experiment it was necessary to do a research of previous studies that focused on measuring pupil size and its variability and studies of measuring pupil size together with cardiac activity.

6.1 A novel method of inducing endogenous pupil oscillations to detect patients with unilateral optic neuritis

Title: A novel method of inducing endogenous pupil oscillations to detect patients with unilateral optic neuritis

Authors: Lamirel C, Ajasse S, Moulignier A, Salomon L, Deschamps R, Gueguen A, et al.

Publication: PLoS ONE 13(8), 2018

Cedric Lamirel et al [6] describe in their study a method of inducing endogenous pupil oscillations to detect patients with unilateral optic neuritis. The study presents fast and easy method, and also describe well monitoring of the pupil parameters. During history pupillary responses have been studied because they could provide reliable markers of optic neuropathies. Abnormal pupillary light reflex including alterations of the latency and amplitude can be found in many neuropathies as glaucoma, optic neuritis or retinopathies. In the previous studies were investigated the characteristics of the pupil cycle time (PCT) to try to find whether it provides a quantitative marker of optic neuritis (ON). Periodic cycles of dilation and constriction were induced by illuminating the pupil using a thin beam of a slit lamp. They found out by measuring the peak to peak time that the PCT is stable, repeatable and significantly longer for patients with ON.

In this study authors present a simple and novel method which is avoiding some of the limitations from previous experiments. The method consisted on biofeedback experiment, where the stimulus luminance was computed during all measurement from the pupil size at each moment. An increasing pupil size induced an increase of the stimulus luminance that turned on a constriction of the pupil and then produced a luminance decrease and so on. . If the transmission delay trough the optic nerve was very short, pupil oscillations would not occur because the system would converge to stable pupil and luminance. But in this case it was working as the frequency of oscillation was approximately 1 Hz. The patients with ON has, at least in part, demyelinated fibres of the optic nerves so in the study was expected lower frequency of pupil oscillations.

For this study 44 subjects in total were tested: 22 patients with history of unilateral ON and 22 healthy participants as reference. Eye data were recorded by Eye-Link II with sampling frequency 250 Hz. Every experiment started with pupil calibration to determinate the maximum, medium and minimum pupil size of each participant. The calibration process is done by displaying 3 seconds of the lowest luminance, 3 seconds of the medium luminance and 3 seconds of the maximum luminance.

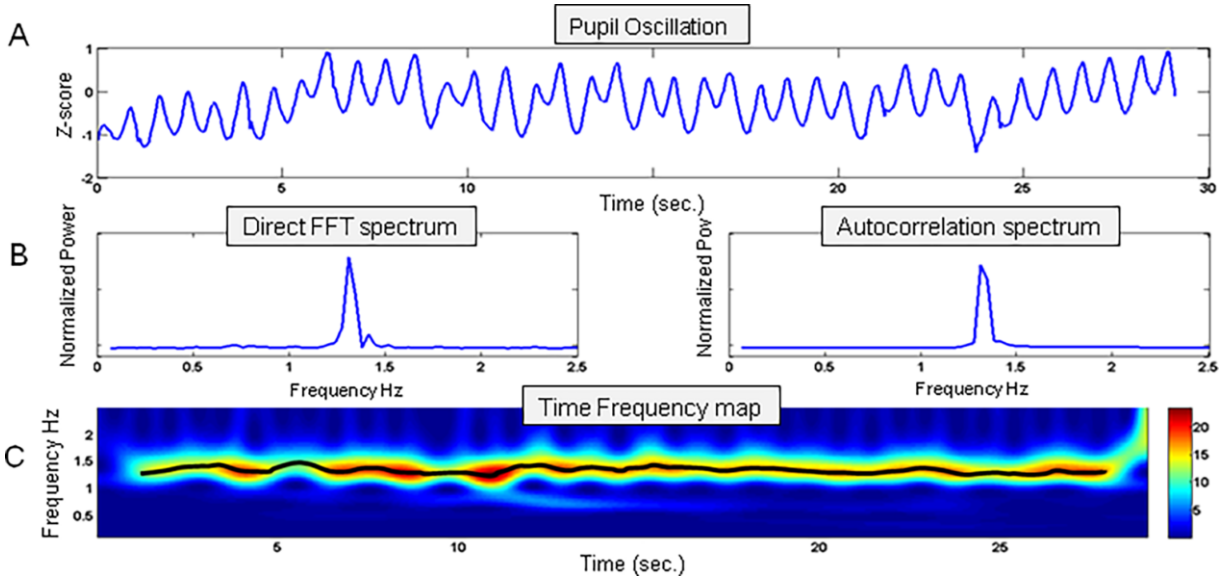

Figure 10: Example of data analysis of pupil oscillation [6]

To analyze the pupil data was used Matlab (example data shown in Figure 10). First were detected blinks and replaced by a linear interpolation. Then was computed a z-normalization. To analyze frequency parameters was used FFT, autocorrelation and FFT of the auto-correlograms. Based on previous studies they concentrated analyses on frequencies with maximum power from 0.5 to 2 Hz.

A statistical analysis with group and affected eye as main factors indicated that pupil oscillation peak frequency between 0.5 and 2 Hz was importantly lower in the affected eye of ON group compared to fellow eye of the group and both eyes of control group. No significant difference was found on fellow eye of ON group and control group.

6.2 Simultaneously measured pupillary light reflex and heart rate variability in healthy children

Title: Simultaneously measured pupillary light reflex and heart rate variability in healthy children

Authors: Daluwatte C, Miles J H, Yao G

Publication: Physiological Measurement 33(6), 2012

In this study Daluwatte et al [7] investigate the potential interrelationship between papillary light reflex (PLR) and heart rate variability (HRV) which are two measures of autonomic nervous system (ANS).

The possible interrelationship between PLR and HRV has been studied recently by several authors and correlations were found in adults during exercise and in patients with acute schizophrenia. The authors of this study measured simultaneously the PLR and HRV in healthy 8-16 years old children. A binocular pupilogram recording system with frame frequency 115 fps was used for measuring the PLR. The light stimulus was provided with a 530 nm green LED. The stimulus pulse width was 100 ms.

Each experiment starts with heart rate recording 5 minutes before the PLR test starts to acquire a baseline reference. The stimulus intensities in a light adapted condition were 69.3 cd/m^2, 872.1 cd/m^2 and 8721.1 cd/m^2. In dark adapted condition the stimulation intensity was 63.1 cd/m^2. First the left eye and then the right eye was stimulated for each test condition. The measurements were repeated four times for each condition with 20 second pause between. Heart rate was recorded during the PLR test and stopped 5 minutes after its completing.

Figure 11: Design of the test procedure [7]

PLR parameters obtained from both eyes during all measurements were averaged to calculate the mean value for each given condition. HRV was evaluated using time and frequency domain. Frequency domain power spectrum was analyzed by using Fast Fourier Transformation (FFT). To evaluate potential effect of the PLR test on HRV parameters, HRV was analyzed in five testing phases. To examine the effects of test conditions, gender and age was used the analysis of covariance of PLR and HRV parameter. To investigate how could be variations in PLR explained by a variations in HRV they used multilinear regression.

The HRV parameters obtained during test phases were very correlated between each other. The correlation of frequency domain HRV in different testing phases was lower than the time domain parameters. The significant correlation between simultaneously measured PLR and HRV parameters has not been proven. The PLR parameters gotten in this study were similar to those reported previously. The mean high frequency was smaller than expected in boys younger than 11 years, but probably it is because of the supine position during measuring. The subjects were divided in two groups: 43% of participants were examined in the morning and the rest in the afternoon. It was studied possible effect of different time of day. No significant difference was found. The HRV parameters in frequency domain were obviously affected by the PLR testing. The results indicates that high frequency decreased 27.3% after test starts. The PLR test may induce a psychological stress and cause decrease.

The authors of this study concluded that variations of PLR and HRV were not associated in healthy children. Nevertgeless, these two variations can provide complementary assessment of different parameters ot the autonomic nervous system.

6.3 Preliminary investigation of pupil size variability: toward non-contact assessment of cardiovascular variability

Title: Preliminary investigation of pupil size variability: toward non-contact assessment of cardiovascular variability

Authors: HUNG Kevin, Yuan-Ting ZHANG

Publication: : 3rd IEEE/EMBS International Summer School on Medical Devices and Biosensors, 137-140, 2006

In the study of Hung and Zhang [8] try to find an effect of exercise on pupil size variability (PSV) spectrum and investigate a way of PSV monitoring. The motivation is to design non-invasive and non-contact method to measure properties of pupil. Monitoring of pupil size and pupilary light reflex is used in novel techniques for detecting narcolepsy, Alzheimer's disease, schizofreny or diabetes. However the characterstics of pupil size variability, which means continuous fluctuation of pupil, is not described exactly. Recent studies have discovered the presence of heart rate variability (HRV) and blood pressure variability (BPV) frequency components in PSV.

In previous studies was performed analysis of power spectral density of HRV to find relation in autonomic balance. High frequency (HF) of the signal, between 0,15-0,5 Hz is associated with parasympathetic activity, while low frequency (LF) between 0,04-0,15 Hz is related to both autonomous systems. It was discovered that exercise increases normalized low frequency power and reduces normalzed high frequency power of HRV. The aim of this study was to verify whether similar phenomena can be find in PSV spectrum.

ECG, respiration effort and finger arterial pressure were measured together with capturing video of pupil. Ambient illumination was kept constant during the experiment. The schema of the experiment is show on Figure 12. The experiment consisted on 6 parts, first measurement was done at rest, the other measurements were taken after 5 minutes of exercise. The subjects were asked to breathe at 12 cycles per minute, that was controled by a metronome signal. The recorded frames of pupil were processed using Canny edge detection. Blinks were removed and data were interpolated by cubic-spline interpolation. Ten healthy subjects were tested. Reduction in normalized high frequency of PSV after excersice and increase during recovery was observed in seven subjects.

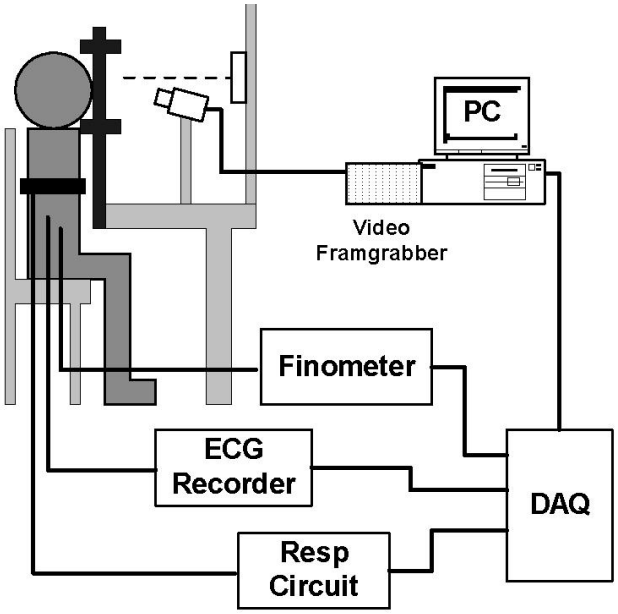

Figure 12: Schema of measuring ECG, respiration, arterial pressure and pupil [8]

According to the results of this study PSV spectrum is affected similarly as HRV spectrum. This finding indicate that PSV could be a valid indicator of cardiovascular activity, but there are required more tests for confirmation with improved exercise protocol. Further analysis of PSV in time-domain, statistics or wavelet analyses could reveal relationship between PSV, HRV and BPV.

6.4 Edge light pupil cycle time

Title: Edge-light pupil cycle time

Authors: Miller S. D, Thompson H. S

Publication: British Journal of Ophthalmology, 62(7), 1978

Miller and Thomspon in their study [9] described the method of generating pupil oscillations. The pupil oscillations are timed and it is realized a measurement of edge-light pupil cycle time. The method consist on illuminating the pupil maring by a thin slit-lamp beam.The teste subject is comfortably seated at the slit lamp in dark room. A 0.5 mm thin slit beam of light is directed to the iris at the inferior limbus and then continues until overlaping the margin of the pupil. When the beam of light enter to the retina, the pupil react by constriction to prevent the light enter. After pupil dilatation the light do not enter and it cuase the pupil increases. Repeting of this cycle generates persistent oscillation. The examination technique is described also in Figure 13.

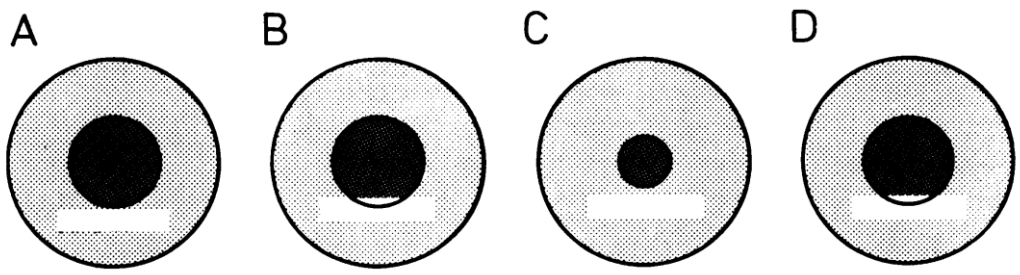

Figure 13: Change of the pupil size induced by beam of light [9]

The authores chosed line stimulus rather than a spot to give a greater and symmetrical stimulus visible to tu pupil. As the basic pupil size is different for each subject it is necessary to make changes in beam elevation to avoid the beam enter to tu the constricted pupil. Quick blink are not interruting the oscillation, but longer do, so the subjects ase asked to blink as little as possible.

In previous studies the results were presented in frequency, cycles per minute. But in this study authors measured time of completion of one loop of the pupil reflex. The results were expressed in milisecond and it were called edge-light pupil cycle time. In the study were tested 58 persons (21 males, 37 females). The effect of different variables was analysed by linear regression for continous variables and for categorical variables was used analysis of variance.

The authors used statistical analyses to obtain results. It was found significant but small trend of increasing cycle time in older subjects. Because of small number of older subjects, subjects ober ře years were restricted from the analysis. The mean pupil cycle time was 882 ms and the standart deviation was 69 ms. According to the measured data only 5% of normal population have a pupil cycle

time longer than 954 ms. The pupil cycle time was not significantly affected by iris colour, visual acuity, sex or other tested variables. In the previous studies was described a tendency of prolonging pupil cycle time after light adaptation, but this trend was not found in this study.

Pupil cycle time can be delayed by many factors. Itesity and frequency of the afferent nerve impulses, the synaptic delays, delays in the efferent nerve path or any slowness of the iris muscles. Previous studies the patiens with multiple sclerosis has prolonged pupil cycle time, that can be caused by damaged conduction of the impulses in the optic nerve.

Measuring of the pupil cycle time represent a fast and simple method for clinical testing of the optic nerve function. The advantge is that ech eye can be evaluated individualy.

6.5 Cardiovascular Autonomic Rhythms in Spontaneous Pupil Fluctuacions

Title: Cardiovascular Autonomic Rhythms in Spontaneous Pupil Fluctuacions

Authors: Calcagnini G, Lino S, Censi F, Cerutti S

Publication: Computers in Cardiology 1997

In this study [13] authors were finding relationship between spontaneous pupil size fluctuations and autonomic rhythms of cardiovascular activity. Spontaneous fluctuations of the pupil diameter (SPDF) withnout any external stimulation have been already reported in previous studies, when the SPDF were identified synchronized with respiration. Short term recording has two main harmonic components: high frequency (0.15-0.4 Hz) synchronous with respiration and low frequency (0.004-0.15 Hz). These frequency rhythms are measured over many nervous pathways. The purpose of the study was to observe these rhythms in spontaneous pupil diameter fluctuations.

For the experiment were recruited 10 adult subjects. They were asked to look at the panel in front of them and the breathing was controled at 15 breaths per minute. During the experiment was recorded ECG, blood pressure and respiration. The pupil images were captured by a CCD camera installed on light helmet. To synchronize the 2 measuring systems it was sended triggering signal. The measurement systems is shown in the schema from Figure 14.

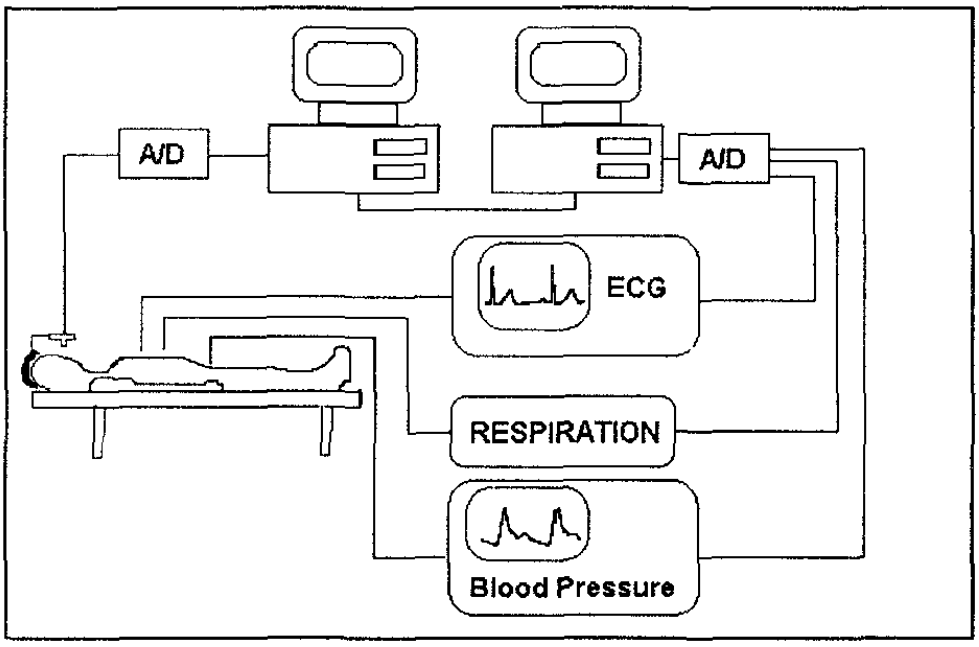

Figure 14: Schema of the system for simultaneous recording [13]

Tachogram, respirogram, systogram and pupilogram were analyzed together with their power frequency spectrum (see in Figure 15). The high frequency at 0.25 Hz represents a respiration. There

were detected also components in LF band in the power spectrum of pupilogram. The finding that the SFPD components are corresponding to LF and HF rhythms suggest the possibility of using this method as non invasive monitoring of autonomic nervous system function.

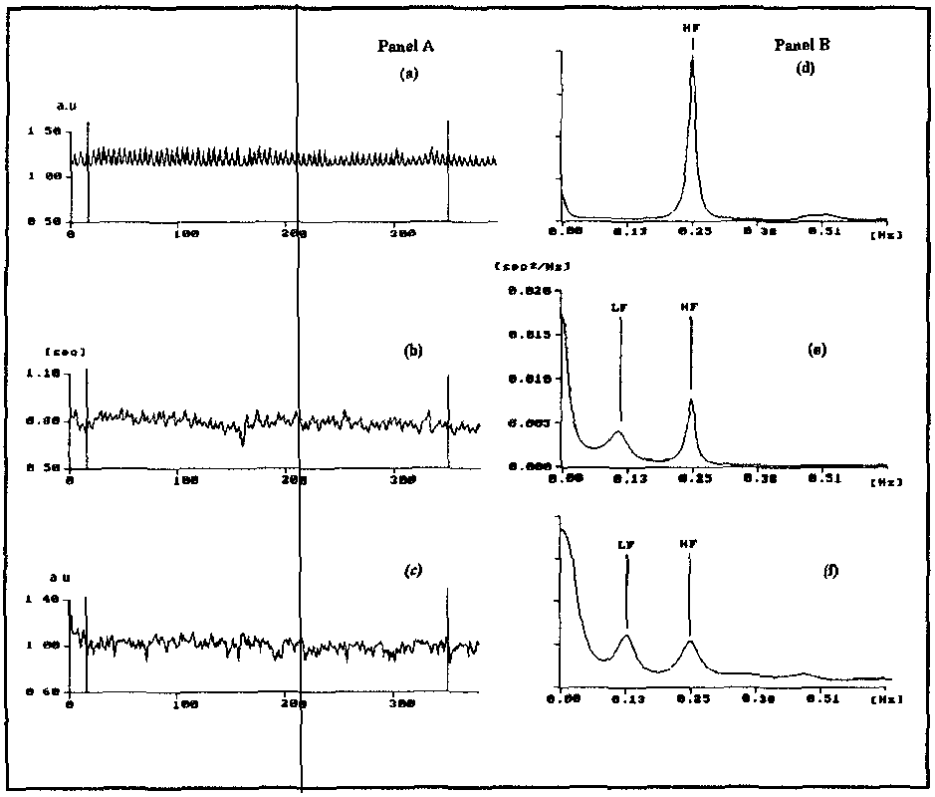

Figure 15: Respirogram (a), tachogram(b) and pupilogram (c) with their power frequency spektrum [13]

7 Measurement of pupil oscillation

To evaluate the variability of pupil diameter it was chosen as the most suitable method monitoring pupil oscillations. The pupil oscillation is obtained using a biofeedback experiment where the luminance of the stimulus is controlled by current pupil size. When the pupil is dilated luminance of the stimulus increase, this leads to narrowing of the pupil and then again luminance decrease (described in Figure 16). Repetition of this cycle creates pupil oscillations. The frequency of pupil oscillations seems to be a good indicator of the condition of nervous system. In previous studies it was demonstrated that the frequency was significantly lower in patiens with optic neuritis.

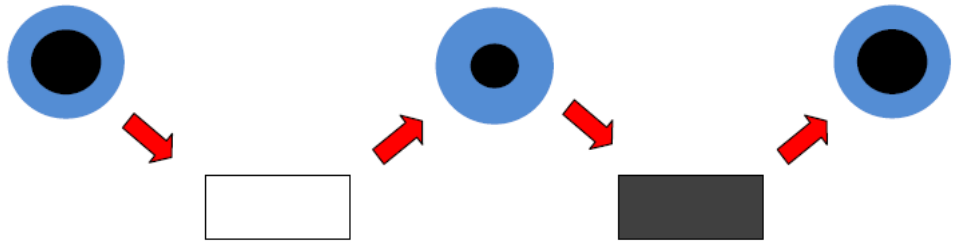

Figure 16: Pupil oscillation elicited by changing luminance of the stimulus

Before designing the experiment we needed to obtain information about variation of pupil size corresponding to different luminance level (ranging from 0 to 255). First it were used different types of stimulus and after analysis we could choose the stimulus with less noisy and more stable results.

The experiments consist on binocular recording pupil size with the eye-tracker Eyelink 1000 Plus with a sampling frequency of 1000 Hz. The experiments were designed in Matlab using Psychtoolbox. The program lets user to change some parameters at the beginning (see in Figure 17): dummy mode, number of trials, trial duration, dominant eye and color of stimuli. The luminance level is changing according to pupil size (of the eye chosen at the beginning of the experiment: we try to choose the dominant eye of the participant, but if it is not possible due to setting problems, we choose the non-dominant eye). In the case of dummy mode turned on, horizontal position of mouse cursor is representing the value of pupil size.

```
%% Dummy mode? yes:1 no:0
dummymode=1;
%
%% Settings
numTrial = 1; %number of trials
trialDuration = 60; %duration of the trial

% CHOOSE DOMINANT EYE
eye=1; % left eye = 1; right eye = 0

% CHOOSE COLOR OF STIMULI
stimCol=0 % grey=0, green=1, red=2, blue=3

%
```

Figure 17: Experimental settings

The photo from Figure 18 shows the experimental instalation where the deskop mount eye-tracker is placed below the display where the stimulus is projected. The chin-rest is placed 50 cm form the camera and it helps to aoid motion artifacts and make the experiments more comfortable for the tested subjects. Each subject was asked for the dominant eye to set in the program the eye for computing luminance of stimulus. All participants were asked to remove their corrective lens if they used any, because it could cause artifacts during eye-tracking.

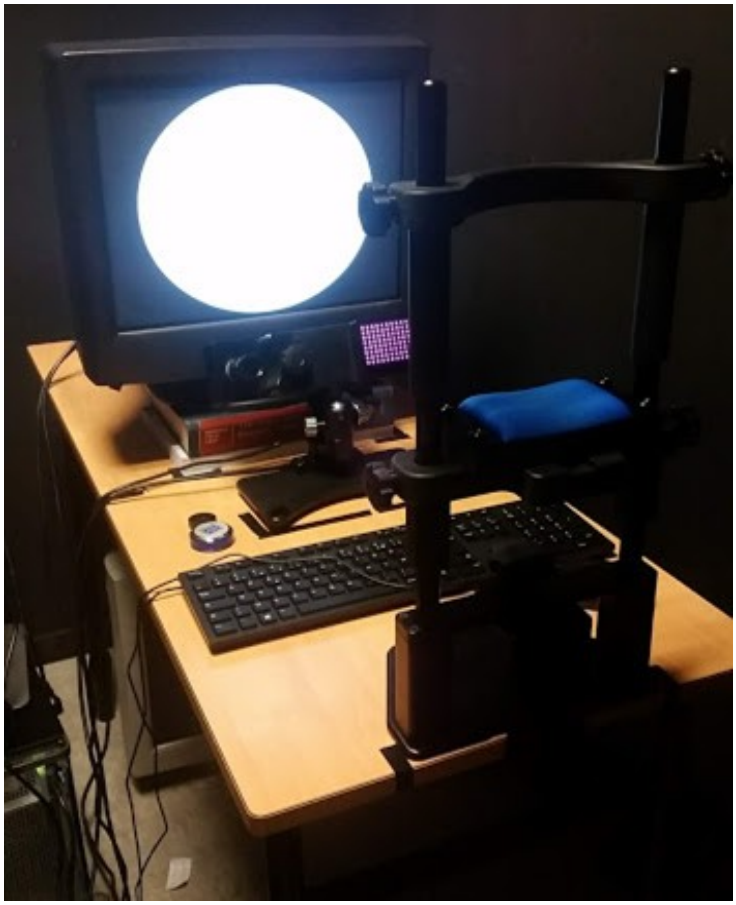

Figure 18: Deskop mount eye-tracker with displayed stimulus and prepared chin-rest

7.1 Stimulus

The stimulus was displayed on monitor with screen resolution of 1280x1024 and 75 Hz frame rate. In the previous experiments we tried different types of stimuli. First we compared stimulus displayed on full screen and disc stimulus of diameter of screen height (Figure 19). The data obtained using disc stimulus appears more stable and less noisy and we decided to continue using disc stimulus for following experiments.

The next task was to to find a constant parameter to provide luminance level from real time pupil size. First we tried to compute the luminance using maximum and minimum pupil size of subject. At the beginning of experiment we displayed for 12 seconds a disc with the highest luminance value (to obtain the minimum size of the pupil) and a disc with the lowest luminance value (to obtain the maximum size of the pupil). The luminance value was computed for real time pupil size using formula:

$$s = \frac{L_{min} - L_{max}}{P_{amin} - P_{amax}} \quad (1)$$

and

$$I = L_{max} - (s * P_{amax}) \quad (2)$$

$$L = I + (s * P_a), \quad (3)$$

where

L …… luminance in digital value

L_{min} …… maximum level of luminance

L_{max} …….... minimum level of luminance

P_a ……..…. real time pupil size

P_{amax} ……..... pupil size corresponding to maximum level of luminance

P_{amin} ……..... pupil size corresponding to minimum level of luminance

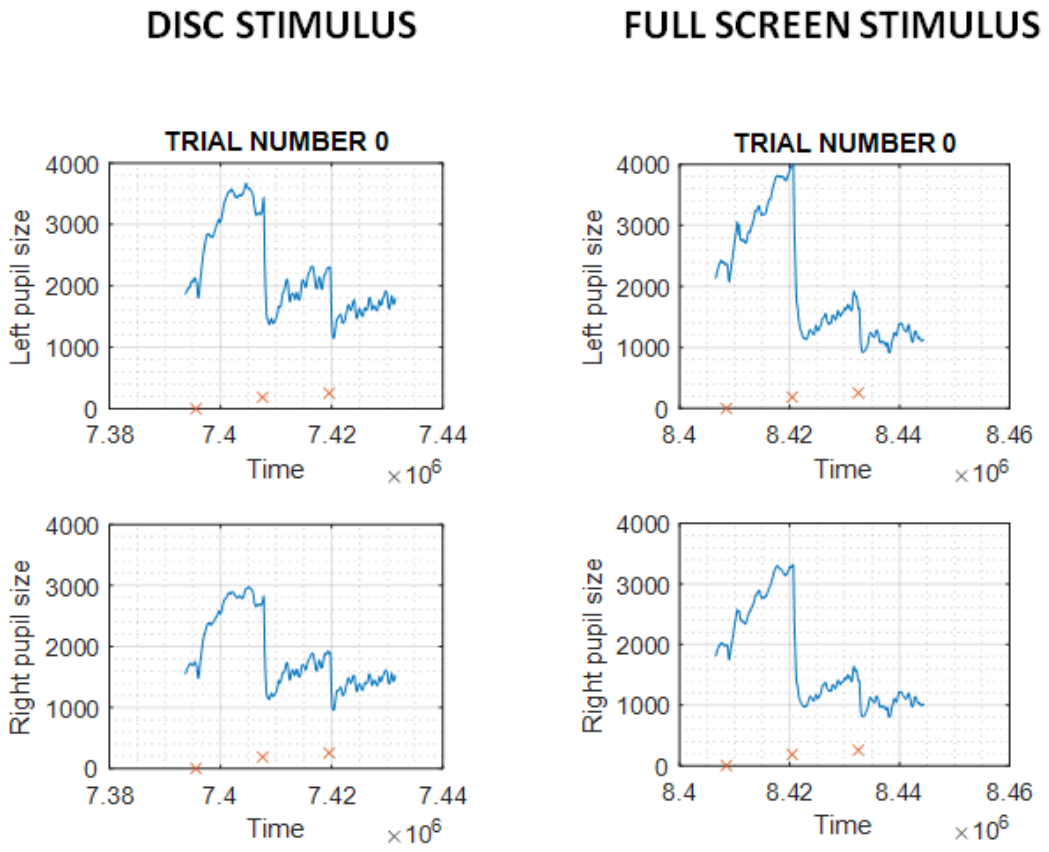

Figure 19: Pupil size of left and right eye corresponding to white, grey and black (maximum, medium and minimum level of luminance), using disc and full screen stimulus.

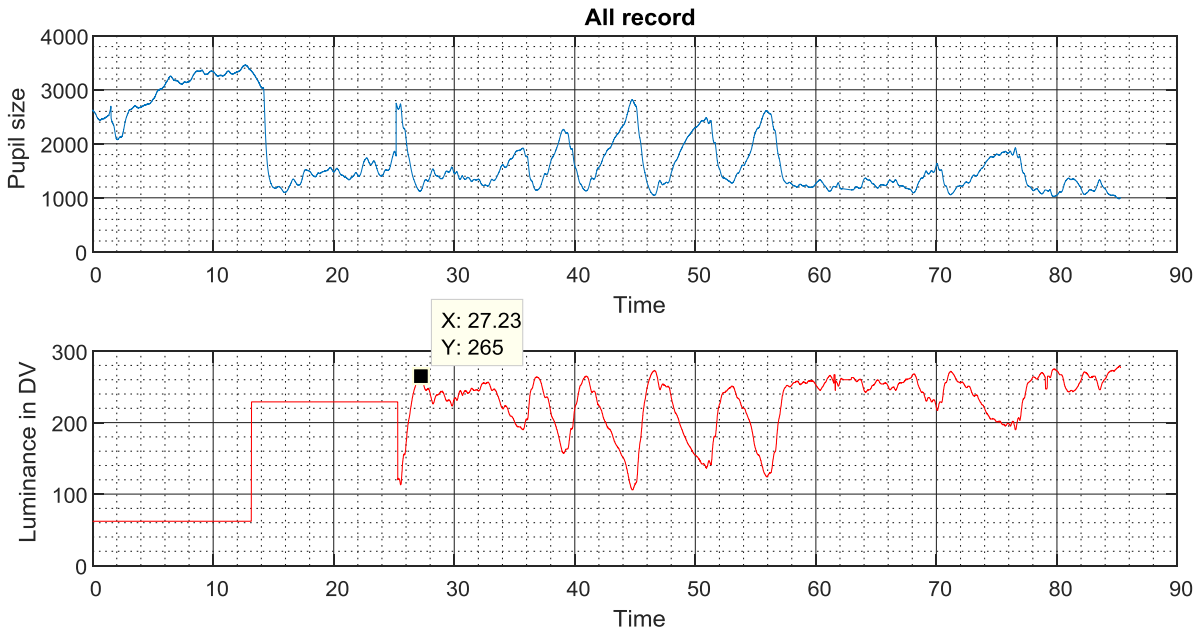

Figure 20: Pupil size and luminance in digital value obtained from the experiment using mentioned formula (1)

In Figure 20 are shown the data obtained from measurement using the formula. First is displayed dark screen and white screen (both for 12 seconds). After starts the trial of duration 60 seconds. It is possible to find some oscillation, but it is not regular and different frequency that expected. The problem was also to set the luminance value to required range (it is clearly visible that the luminance reaches values higher than 255). Due to received results we decided to attempt compute the luminance different way.

In the final experiment we obtain luminance value by dividing real time pupil size by the maximum pupil size of the subject. The stimulus of pupil-contingent experiment consists of a disc of diameter of screen height displayed on a dark background. At the beginning of experiment is displayed for 12 seconds a disc with the lowest luminance value to obtain the maximum size of the pupil. This value is then used to calculate luminance value for each recorded sample of pupil size. To convert pupil size into luminance value we divided real time pupil size by the maximum size of pupil and then the value was set as to maintain the luminance in the 0-255 range. The pupil oscillation obtained this way is shown in Figure 21. After 12 seconds of displaying dark screen starts the trial which duration is 60 seconds. The luminance is computed in function of pupil size of chosen eye.

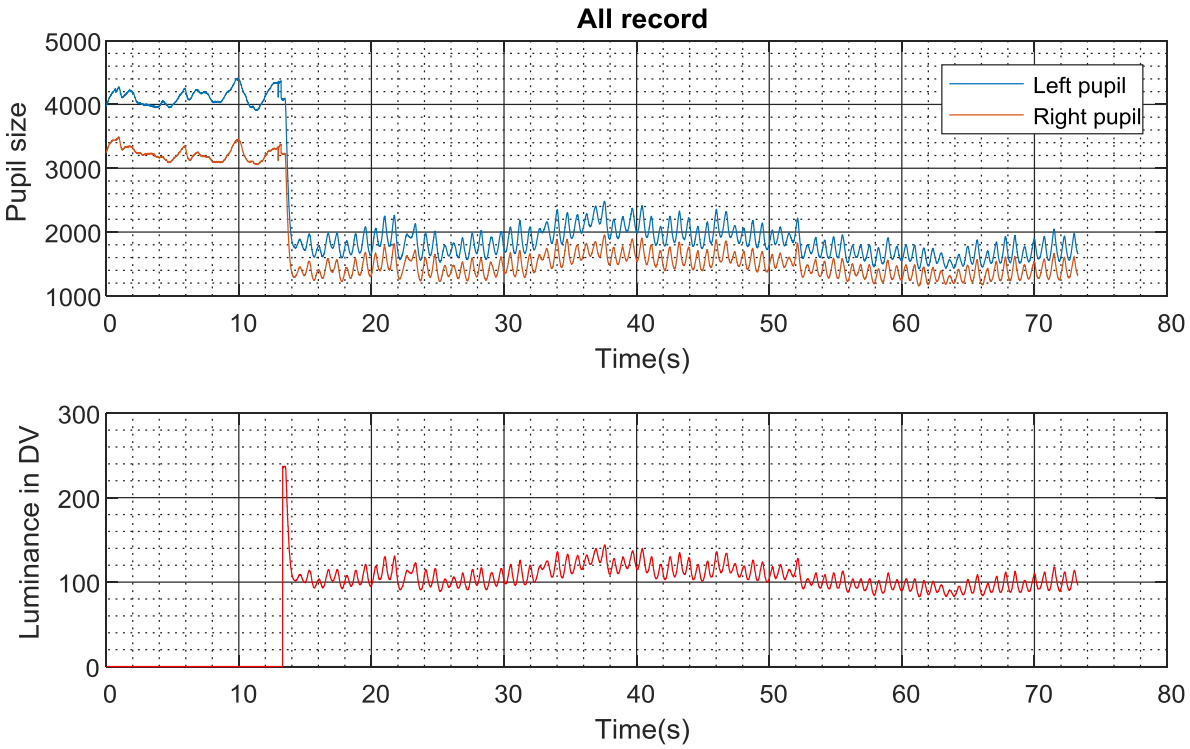

Figure 21: Pupil size and luminance in digital value obtained from the final version of computing luminance

7.2 Data processing

Raw data obtained from the experiment are shown in Figure 22. Raw data contain blinks and pupil size is in pixels. The program for processing data is developed using Matlab. First of all blinks are detected and removed. Values between start and end of each blink are interpolated by linear interpolation. Then a z-normalization is computed, because for the analyses it is not needed absolute value of pupil size. The character of experiment allows to study relative pupil size, it's change during time.

Full record contains two parts. First 12 seconds was displayed black stimulus (stimulus of the lower luminance) to capture the largest size of pupil, which is then used to compute luminance value for pupil in real time. This part is necessary as each subject has different default size of the pupil, the position and angle of the head can be little different every trial and both eyes are in different distance from camera. The second part represent the main measurement which s the biofeedback obtained pupil oscillation. Before processing the data of the pupil oscillation, it was removed the first part, because it could affect the results.

As mentioned above the important information of the signal is in frequency domain. Frequency analysis of pupil oscilaltions is realized using Fast Fourier Transformation. Frequency analyis contains power frequency spectrum and then spectrogram, where we can observe how the frequency changes in time. The important frequency is expected according to the previous studies [6]

between 0,5-2 Hz. In example from Figure 23 the spectrum shows the high frequency of 1,38 Hz and it persist about this value during all the trial. The red cross in time domain represents the mean value of pupil size in the trial.

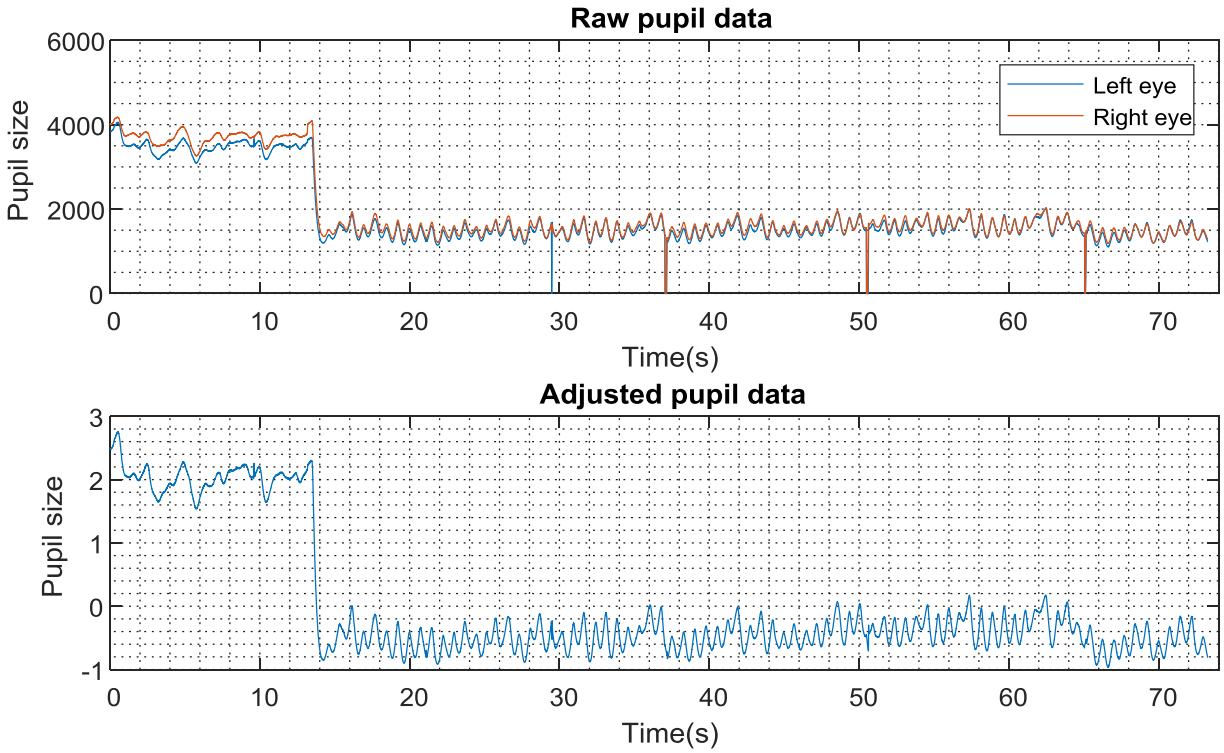

Figure 22: Raw pupil data (above) and pupil data after removing blinks and normalization (below)

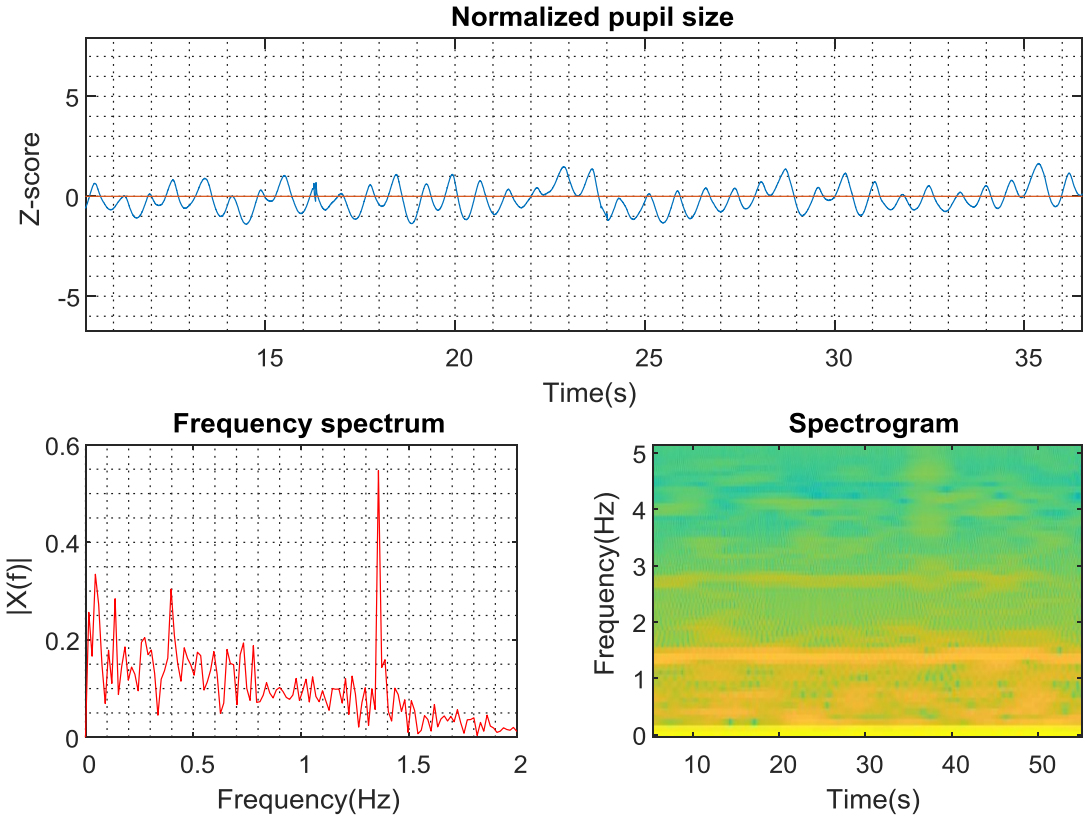

Figure 23: Example of processed pupil data

7.3 Statistical analysis of pupil oscillation

Ten healthy participants with age ranging from 22 to 29 were recruited to test the experiment to obtain pupil oscillations.

The frequency of pupil oscillation ranges from 1.328 to 1.465 Hz. None of the meassurments were identified as a remote observation. The mean frequency of pupil oscillation is 1.369 Hz with standard deviation 0.046 Hz. According to the value of variation coefficient (3.5%) the analyzed group cannot be considered homogenous.

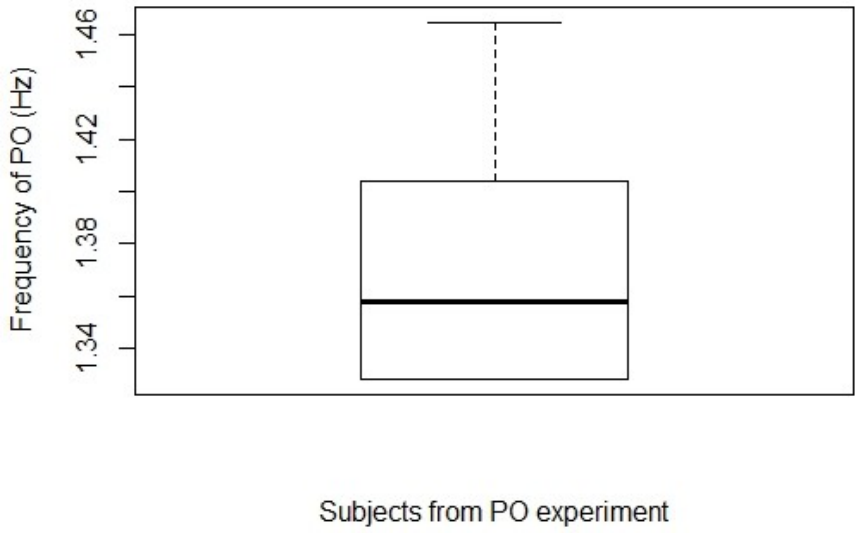

Figure 24: Box plot of distribution of PO frequency

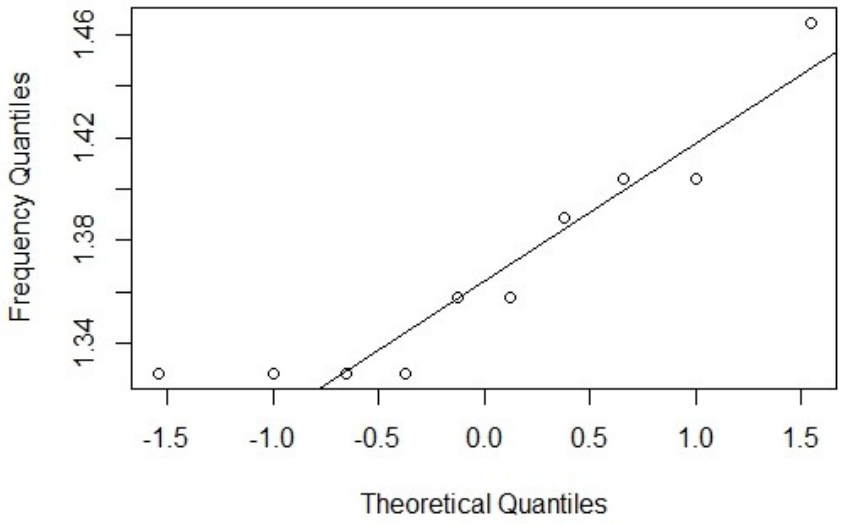

Figure 25: Normal Q-Q plot of PO frequency

Based on the graphical representation (see on Figure 24 and Figure 25) and selective skewness and sharpness (both lie in the interval (-2,2)), it is expected that the highest frequency of PO of the tested group has normal distribution. According to the 3 sigma rule, approximately 95% of participants should have the pupil oscillation frequency from 1.231 to 1.507 Hz. The normal distribution is confirmed by the Shapiro-Wilk test of the normality (p-value=0.065).

As the group of the data meet the conditions of normality it can be used single sample t-test. The test is used to compare the pupil ocillation frequency with the normal heart rate frequency in adults, which is ranging 60-100 beats per minute [12]. The null hypothesis expects that the frequency of the pupil oscillation should be at the same range as the heart beat frequency. In normal adults the heart rate frequency is from 1 to 1.6 Hz. The null hypothesis says that the mean frequency of the pupil oscilaltions is same as the mean frequency of heart rate in normal adults.

$$\textit{Null hypothesis: } H_0 = 1.3 \textit{ Hz}$$
$$\textit{Alterntive hypothesis: } H_A \neq 1.3 \textit{ Hz}$$

It was used a single sample t-test and the null hypothesis was rejected (p-value = 0.001). The mean frequency of pupil oscillation in heatly adults is expected 1.369 Hz. With 95% of reliability the frequency of pupil oscillation is found between 1.336 and 1.401 Hz.

According to the statistical analysis the frequency of hear rate and pupil oscillations in adults are not at the same values. The mean frequency of PO is little higher that the expected frequency of HR. The heart rate frequency was estimated from theoretical knowledge. The measuring of pupil size and cardiac activity could obtain more precise data for evaluation.

8 Measurement of POs together with cardiac activity

In the next part of the thesis we focused on measuring pupil oscillations together with cardiac activity. Measuring of this two signal synchronized together is important for the comparison of their characteristics in time and frequency domain. It was measured electrocardiogram (ECG) which allows computing heart rate variability later. First we tried to measure ECG using a chest strap Zephyr. However this method was prone to motion artifacts, the installation was uncomfortable (specially for women) and the synchronization with eyetracking measurement was difficult and not precise enough. Because of these issues we decided to choose another device for ECG measurement.

Figure 26: BIOPAC MP150 with ECG100C and UIM100C module, front side (left), back side with digital input for trigger (right)

To record ECG we used BIOPAC MP150 with the ECG100C Electrocardiogram Amplifier and the UIM100C module. Complete device is shown in Figure 26. Reusable suction cup electrodes were used for one lead measurement. To synchronize the measurement with eyetracker a synchronization signal was sent to trigger of Biopac. The program designed in Matlab which controls projection of stimulus and lauching of the eye-tracker send zero to the trigger. Then from trigger the signal is led to channel 8, where the default value is 5 V. Every measurement has part of adaptation pupil to dark (first 12 seconds) and the measurement of pupil oscillation. Because of that during each experiment the synchronization signal is sent two times. In Figure 29 is shown whole record of one subject. ECG recording starts when the instalation of electrodes is done and the subject is ready for eye-tracking measurement. Before start of pupil measurement the calibration of eye-tracker is needed.

The ECG data are provided as text file by acqKnowldge software. Before running experiment it is needed to set up channels to plot ECG and trigger value in time (Figure 27) and set up trigger (Figure 28) in the acqKnowledge.

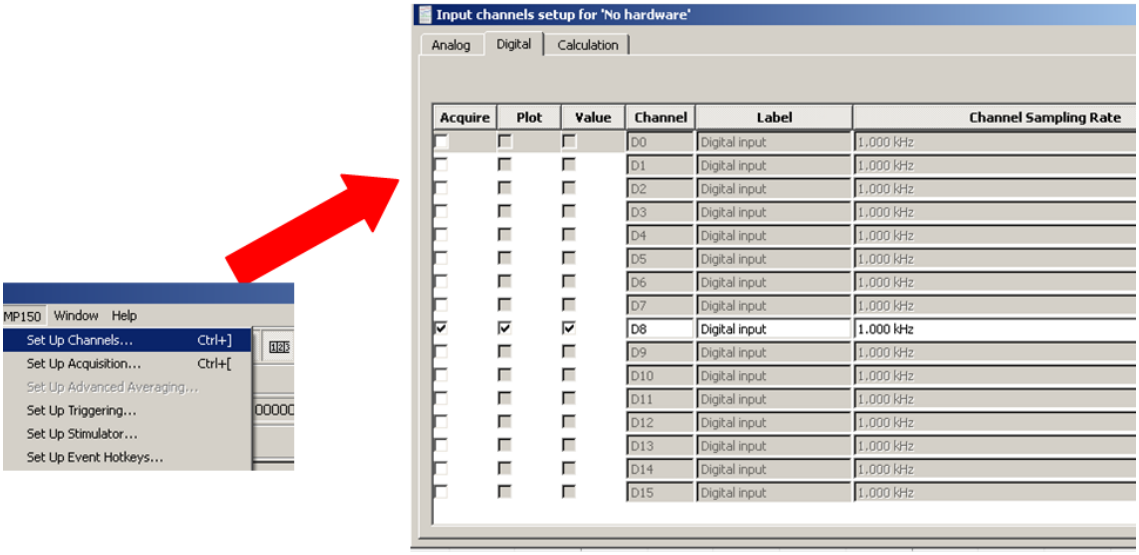

Figure 27: Set Up Channels in acqKnowledge

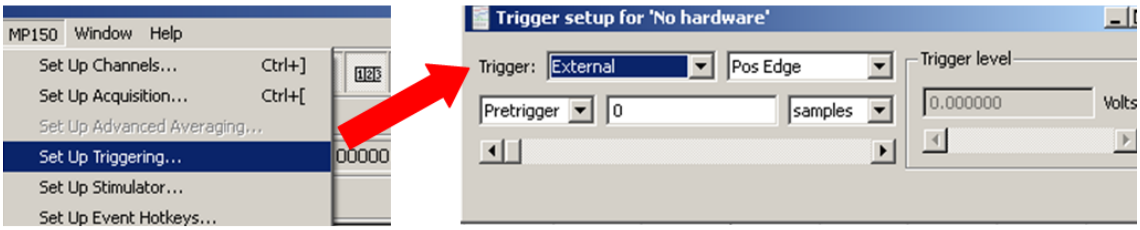

Figure 28: Sett Up Triggering in acqKnowledge

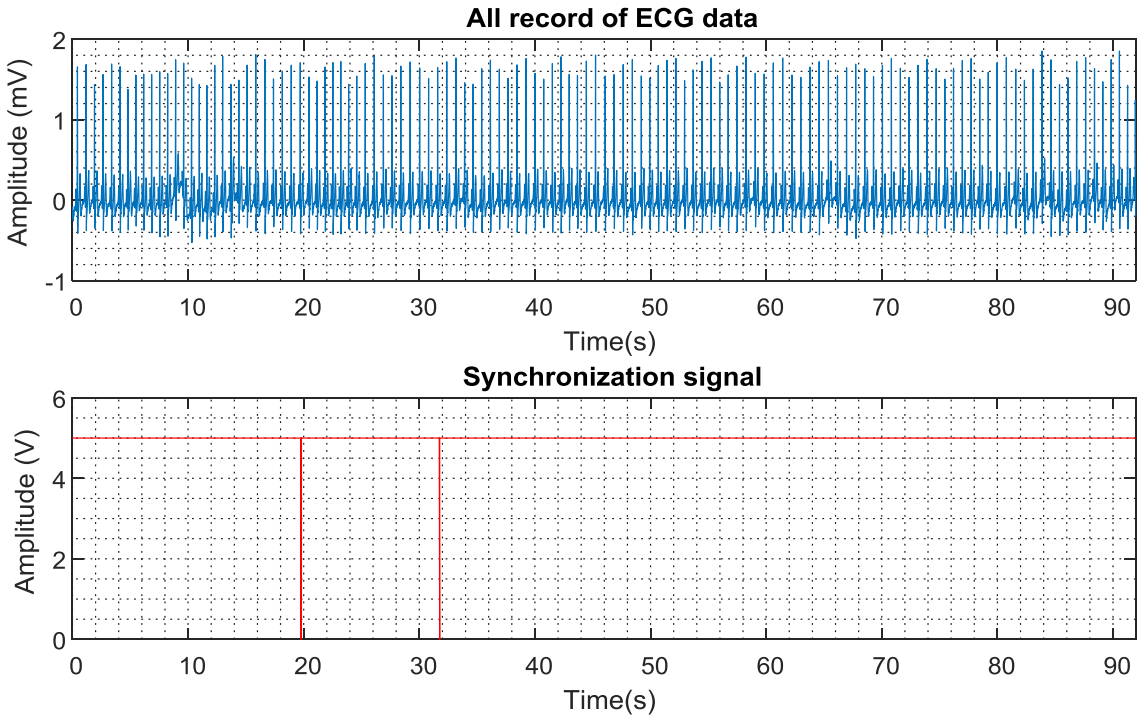

Figure 29: ECG data together with synchronization signal

Schema on Figure 30 describes the design of final experiment which includes measurement of pupil data and cardiac activity. Disc stimulus with luminance corresponding to current pupil size is displayed on the screen in front of subject. Subject's head is resting on the chin-rest and subject is asked to focus on the red cross in the centre of the disc. This eliminates motion artifacts. Current pupil size is recorded during all experiment using eye-tracker Eyelink 1000 Plus. Luminance value at each moment and pupil data are saved for further processing. ECG is measured since the electrodes are installed well. For measuring ECG it is used II-lead which provide large R-peaks to calculate heart rate variability (HRV) later. The ECG data are provided by acqKnowledge software, sent to the computer and saved. Synchronization signal is sent via serial port.

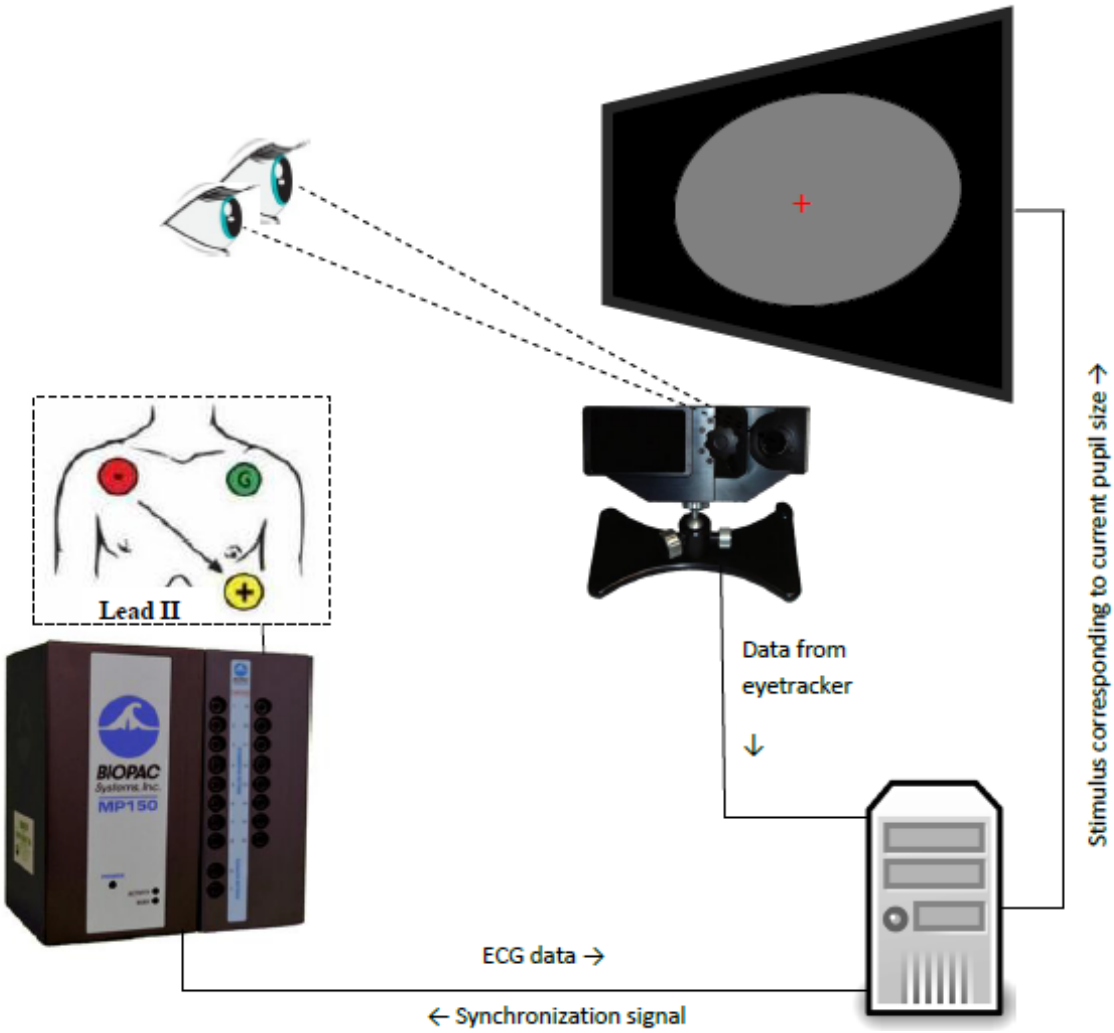

Figure 30: Schema of synchronized measurement of POs and ECG

8.1 Data processing

For analysis the pupil and ECG data obtained from the experiment described above was designed an application where the signals are time synchronized (shown in Figure 35). The data from eye-tracker are inputted as an m.file and they contain information of the pupil size and luminance during the experiment. Before plotting the data the first part of dark accommodation is deleted as the important information of pupil oscillation is in the main part of the experiment. Then the blinks are detected and deleted. The missing data between start and end of each blink are interpolated by linear interpolation. As mentioned before the analysis is not dedicated to abolute pupil size, so the pupil size is normalized by z-normalization to plot it's change in time. ECG data are inserted as a text file which is provided by acqKnowledge software. Whole record is cut according to synchronization signal. The luminance, normalized pupil size and ECG are presented in time domain and they are synchronized in time.

8.2 Detection of hear rate and HRV

The analysis of ECG contains computing heart rate (in beats per minute) and its variability in time. The heart rate is done by determining the highest values in amplitude as the peak R. The heart rate is then determined from R-R intervals. For this is used in Matlab a function findpeaks(x,y,'MinPeakHeight','MinPeakDistance'). The heart rate variability presents changes of heart rate in time. It is computed according to the distance between each R-peaks. As the subjects were tested in rest it is not expected large variability.

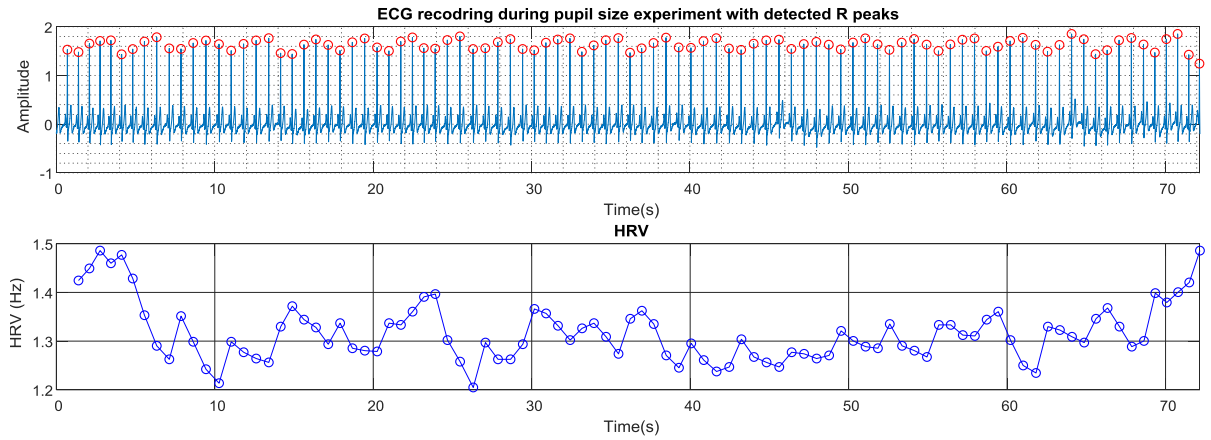

Figure 31: ECG and computed HRV

8.3 Pupil oscillation and its variability

The pupil oscillations are detected similar way as heart rate. The peaks of every cycle are detected and the number of oscillations per minute is calculated. The varibility depends on the distance between peaks. It can be little distorted due to the long blinks. Short blinks could not affetc the variability after deleting, but long blinks can cause the change of the luminance of stimulus during experiment and then affect the oscillation. In Figure 32 is shown pupil oscillation variaility and heart rate variablity of one subject from one measurement. The variability of pupil oscillation appears less stable, the values out of the expected range can be result of blinks, where the luminance of stimulus changed.

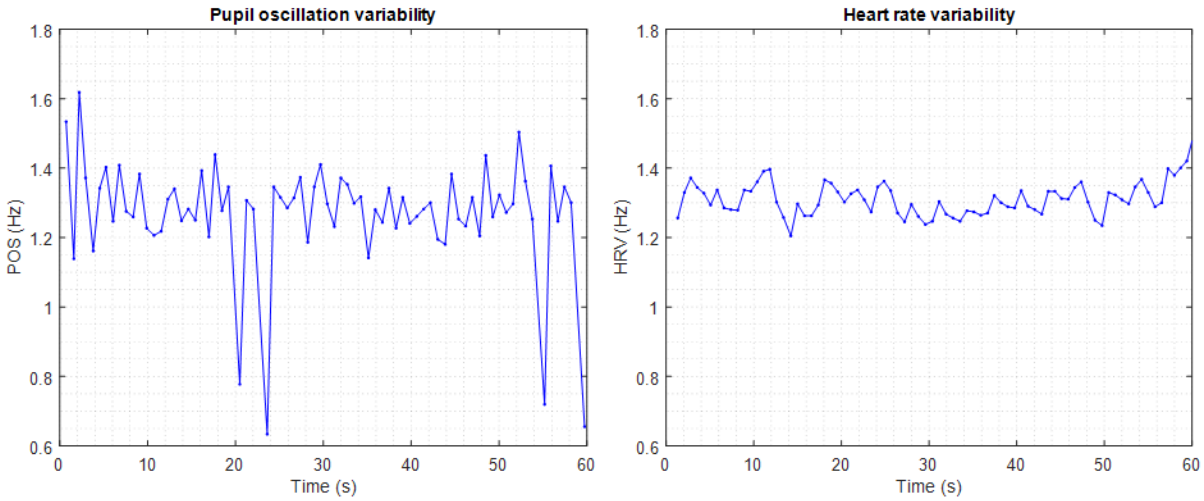

Figure 32: Pupil oscillation and heart rate variability of one subject

8.4 Frequency analysis

The frequency analysis allows to represent the signals in frequency domain. For the presentation was chosen the power frequency spectrum and spectrogram. Both are used for the ECG signal and pupil size signal to have the opportunity of comparing the signals in frequency domain.

8.4.1 Power frequency spectrum

In Figure 33 is the example of the code for computing frequency spectrum. For the conversion of the signal from the time domain to the frequency domain is used the Fast Fourier Transfrmation (FFT). There is a function in Matlab for FFT. The used imput frequency is the sampling frequency of eye-tracker in case of pupil data and of BIOPAC in case of ECG data.

```
case 'Frequency spectrum'
    Fs = 1000; % sampling frequency
    T = 1/Fs; % period
    L = length(leftPupil1);

    NFFT = 2^nextpow2(L);
    leftX = fft(leftPupil1,NFFT)/L;
    f = Fs/2*linspace(0,1,NFFT/2+1);

    axes(handles.axes3); % plot the spectrum
    plot(f,2*abs(leftX(1:NFFT/2+1)),'r');
    xlabel('Frequency (Hz)');ylabel('|X(f)|');grid minor;
    xlim([0 2]);ylim([0 1]);
```

Figure 33: Example of code in Matlab for drawing frequency spectrum

8.4.2 Spectrogram

Spectrogram represents the signal in time-frequency domain. That allows observe how the signal parameters change in time. The example code for computing spectrogram is in Figure 34. There is Matlab function for drawing spectrogram. It is needed to input sampling frequency of used device (eye-tracker and ECG device) and choose window size. The window size is selected according to the length of the recorded signal.

```
case 'Spectrogram'
     Fs = 1000; % sampling frequency
    T = 1/Fs; % period
    L = length(leftPupil1);
    leftZdec=decimate(double(leftPupil1),20);  % subsampling
    Fs_nv=Fs/20;
    Lspectro=400; % Window size
    NFFTspectro = 2^nextpow2(Lspectro);
    [S,F,T,P] = spectrogram(leftZdec,Lspectro,Lspectro-1,NFFTspectro,Fs_nv);

    axes(handles.axes3);
    imagesc(T,F,log10(P)) % it is better to use a log plot for visualisation...
    axis xy; ylabel('Frequency');xlabel('Time(s)');
```

Figure 34: Example of code in Matlab for drawing spectrogram

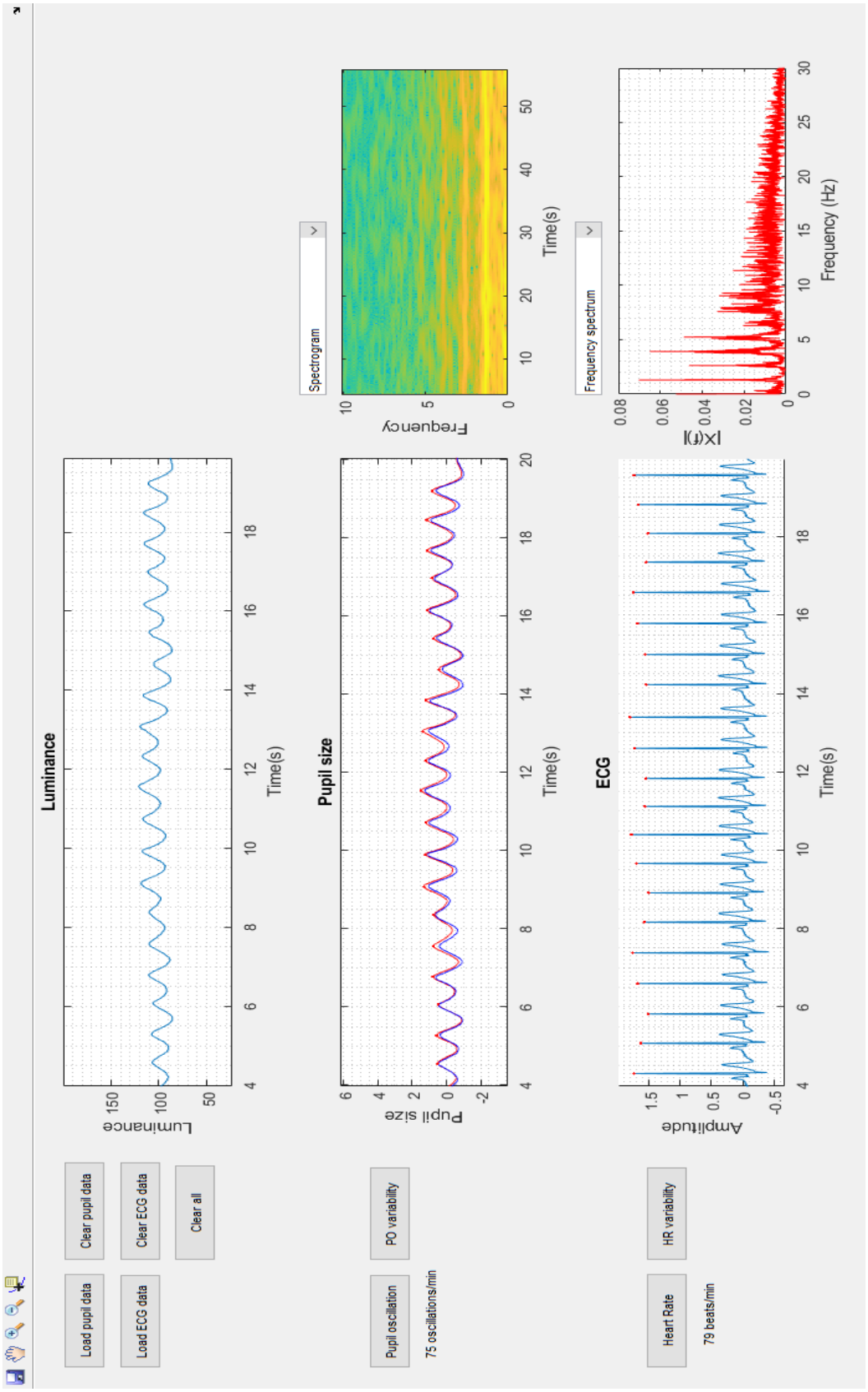

Figure 35: Application for data analysis

9 Conclusion

The diploma thesis was dedicated to measurement of the pupil size together with cardiac activity to find a relation between these two signals. It was designed a biofeedback experiment which allows detection of the pupil oscillation. The experiment was based on eye-tracking and changing the luminance of displayed stimulus according to actual pupil size. When the pupil size increase, the luminance value increase too and it makes a dilatation of the pupil and decreasing of the luminance. Repetition of this cycle generates pupil oscillations.

The pupil oscillations were measured in ten healthy adults and the frequency characteristics were analyzed. The mean value of the frequency was found in 1.369 Hz. The time-frequency analysis shows that the pupil oscillations maintain similar value for each person during all the experiment. The comparison of pupil and cardiac activity was done using theoretical values of heart rate. If the heart rate is expected between 1-1.6 Hz, we can find similar value of mean frequency in HR and POs.

In the next part of the thesis the ECG measurement was added to the experimental protocol. Electrical activity of heart was measured by one lead ECG, time synchronized with previous eye-tracking experiment. To process the recorded data was designed an application in Matlab. The application presents luminance, pupil size and ECG data, synchronized in time and with deleted blinks. It computes the power frequency spectrum and spectrogram using Fast Fourier Transformation for both, pupil and ECG, signals. The frequency analysis allows compare the signals in frequency domain. Then there is the possibility to calculate pupil oscillations per minute and heart rate. The POs variability and HR variability are then drawn in new figure. The presentation of variability of the signals is another way for evaluation in time-frequency domain.

The application was tested in data obtained from measurement of two subjects. It was used designed experimental protocol which includes measuring of pupil size and ECG. The hypothesis expects strong relation between these two signals as both are innervated by sympathetic and parasympathetic nervous systems. Comparison of PO variability and HR variability points to possible relation as both signals are situated at similar frequencies and are constant during measuring at rest.

The thesis presents the way of comparing the variability of pupil size with cardiac activity. The designed experiment it is suitable for future studies which may include measurement after exercise or psychical load to confirm that the pupil oscillations and heart rate are affected the same way.

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